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Intermittent Standing and Prior Exercise as Strategies to Prevent Arterial Stiffening That Can Arise From Prolonged Sitting

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INTERMITTENT STANDING AND PRIOR EXERCISE AS STRATEGIES TO PREVENT ARTERIAL STIFFENING THAT CAN ARISE FROM PROLONGED SITTING

by

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ABSTRACT

Prolonged sitting can cause adverse vascular responses, including elevations in aortic stiffness which might contribute to cardiovascular disease (CVD). Few studies have investigated the impact of intermittent standing and/or prior exercise as strategies to mitigate these deleterious vascular changes. **Purpose:** to explore vascular health responses to prolonged sitting, with and without intermittent standing and/or prior exercise. **Methods:** 15 males aged between 18 and 31 years were recruited. Participants completed a control condition (Sitting Only), and three randomized strategy conditions (Sitting Plus Standing, Exercise Plus Sitting, Exercise Plus Sitting Plus Standing). For all conditions, measurements of carotid-femoral PWV (cfPWV) were taken at pre- and post-intervention, and brachial and central blood pressure (BP) at pre-, 1-hour-, 2-hours-, and 3-hours-intervention. **Results:** Condition and time did not interact significantly for any vascular parameters. cfPWV significantly increased from pre- to post-intervention for all conditions ($p=0.003$, $p=0.043$, $p=0.001$, $p=0.008$), as well for brachial and central diastolic BP for the control condition ($p=0.044$, $p=0.015$). Brachial systolic BP at 3-hours-intervention and central systolic BP at 1-hour-intervention were significantly higher for the control condition compared to the Exercise Plus Sitting Plus Standing condition ($p=0.005$, $p=0.010$). **Conclusions:** BP and cfPWV may not be meaningfully influenced by these strategies and do not seem to be related. These strategies may reduce BP in the brachial and aortic arteries, and a combination of prior exercise and intermittent standing may reduce systolic BP more effectively. Future research should explore mechanistic links between sitting and aortic stiffening and experiment with other strategies.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ACSM	American College of Sports Medicine
AHA	American Heart Association
BP	Blood Pressure
CVD	Cardiovascular Disease
CF-PWV	Carotid-Femoral Pulse Waive Velocity
CDC	Centers for Disease Control and Prevention
CEC(s)	Circulating Endothelial Cells
DHHS	Department of Health and Human Services
FMD	Flow-mediated Dilation
HDL	High Density Lipoprotein
IRB	Institutional Review Board
LDL	Low Density Lipoprotein
LPL	Lipoprotein Lipase
VO2 max	Maximum Oxygen Consumption
MET(s)	Metabolic Equivalent(s)
PA	Physical Activity
PWV	Pulse Waive Velocity
USM	The University of Southern Mississippi
USA	United States of America

CHAPTER I – INTRODUCTION

1.1 Background

The advancements in health care over the years have led to longer life spans and subsequent increases in chronic disease development (Yusuf et al., 2001). CVD has now been the leading cause of mortality in the United States of America (USA) for over one-hundred years (since 1921), with an average of 655,000 deaths per year and costing 219 million dollars per year in health care expenses (Centers for Disease Control and Prevention [CDC], 2020). The risk factors for CVD and the health benefits of increasing physical activity (PA) for reducing CVD risk have been clearly identified and established in the literature and by health institutions. But the chronic physiological mechanisms that link factors such as sedentary behavior with adverse vascular and blood flow alterations and CVD development are still not completely understood (Park et al., 2020).

The human body distributes blood throughout the periphery based on its demand, with the oxygen requirement of bodily organs and tissues varying based on their current metabolism. Vasoconstriction and vasodilation actively control blood flow to target organs and tissues through the contraction of vascular smooth muscle or the release of nitric oxide from the endothelial linings respectively (Guyenet, 2006). The functioning of these vascular mechanisms is therefore crucial for maintaining homeostatic bodily processes and health throughout the life span. Arterial stiffening is a vascular condition that has been found to cause reductions in blood flow regulation (Zieman et al., 2005). With evidence linking this condition to the development of atherosclerosis and CVD (Mattace-Raso et al., 2006; Mitchell et al., 2010; Niiranen et al., 2016; van Popele et al.,

2001; Zieman et al., 2005), arterial stiffening has now been highlighted as an epidemic (Zieman et al., 2005).

Engagement in PA has been found to have a strong inverse relationship with all-cause mortality, CVD, and other chronic disease incidence (Gardner et al., 2011; Nocon et al., 2008). Current guidelines recommend that healthy adults should engage in 150-300 minutes of moderate PA or 75-150 minutes of vigorous PA per week (or a combination), and muscular strengthening activities twice per week (ACSM, 2016; DHHS, 2018). However, as of 2018, only 24% of the USA population meet both these guidelines (CDC, 2018). Sedentary behavior is also becoming very habitual in the USA, with 7.7 hours (55%) of the average person's waking day being spent sitting (Matthews et al., 2008). This is worrying, with the epidemiological evidence indicating sedentary time to be independently associated with increased risk of all-cause mortality and CVD, regardless of physical activity level (Patterson et al., 2018).

Although the epidemiological consequences of chronic sedentary behavior are clear, the physiological mechanisms linking acute sedentary behavior to adverse chronic health changes are not currently known (Park et al., 2020). Arterial stiffening is likely a contributing mechanism, with this vascular condition being associated with common health markers that are linked with chronic sedentary behaviors including high glucose and insulin levels, dyslipidemia, and damage and functional impairment to the endothelial linings of the microvasculature (Benetos et al., 2002; Demiot et al., 2007; Zieman et al., 2005). Acute sedentary exposures have been found to induce these same metabolic trends as well as reductions in flow-mediated dilation that may be linked with

arterial stiffening and CVD development, but it is still not certain if these acute markers cause these chronic health changes (Paterson et al., 2020).

Preventative strategies for reducing these adverse health changes from acute sedentary exposure are necessary considering that prolonged sitting is often unavoidable for desk and office workers. Interrupting prolonged sitting with moderate and even light intensity PA breaks have been found to be effective for reducing metabolic and vascular markers, however a lack of research has tested the effectiveness of intermittent standing breaks on vascular function (Chastin et al., 2015; Paterson et al., 2020). Replacing sitting time with standing has not been found to have chronic benefits for BP, while little research has measured the effects on vascular changes also (Saeidifard et al., 2020). Performing exercise prior to prolonged sitting would seem beneficial, with the current evidence showing increases in vascular function and reductions in BP that can last for hours after the cessation of PA performance (Carpio-Rivera et al., 2016; Colberg et al., 2016; Green & Smith, 2018). But only two experimentally controlled studies to date have examined the effects of prior exercise, with both finding benefits to flow-mediated dilation (Ballard et al., 2017; Morishima et al., 2017). The purpose of this study was therefore to explore the cardiovascular health response to prolonged sitting with and without interruption with standing and/or prior exercise.

1.2 Specific Aims

This research addressed the following aims:

1. Measured and evaluated the cardiovascular health response of four separate conditions: 1) 3 hours of uninterrupted sitting (condition ‘SO’), 2) 3 hours of sitting plus intermittent standing of 10 minutes every 30 minutes (condition ‘SSt’), 3) 30 minutes of prior exercise at moderate intensity plus 3 hours of uninterrupted sitting (condition ‘ES’), and 4) prior exercise (moderate intensity) plus sitting plus intermittent standing (condition ‘ESSt’). Specific measures included:
    a) Primary variable of carotid-femoral pulse-wave velocity (cfPWV).
    b) Secondary variable central BP.
2. Compared and determined the most effective strategies to prevent arterial stiffening from prolonged sitting
3. Evaluated whether changes in arterial stiffness were associated with cardiovascular hemodynamics, including BP.

1.3 Hypothesis

1. Post intervention measures of BP and cfPWV will be significantly increased from baseline for the ‘SO’ and ‘SSt’ conditions, and unchanged for the ‘ES’ and ‘E+S+St’ conditions.
2. Post intervention measures of BP and cfPWV will be significantly greater for ‘SO’ and ‘SSt’ compared to ‘ES’ and ‘ESSt’.
3. Post intervention measures of BP and cfPWV will not be significantly different between ‘SO’ and ‘SSt’, and ‘E+S’ and ‘ESSt’.
4. ‘ES’ and ‘ESSt’ will have the most favorable vascular outcomes post intervention and effectively prevent the elevations in arterial stiffness and BP that occur from prolonged sitting, while ‘SO’ and ‘SSt’ will not.

1.4 Significance and Gaps in the Literature

CVD incidence and sedentary behaviors are very prevalent throughout the world and have high associations in the epidemiological literature. Sedentary exposures can cause deleterious chronic health changes, but how these changes occur as well as how they are linked to CVD development are not known (Park et al., 2020). Arterial stiffening from acute sedentary exposures could be a possible mechanism as found in recent literature (Paterson., et al 2020; Saunders et al., 2018). This thesis will assess vascular measures of cBP and cfPWV in response to prolonged sitting to explore these findings and add to a growing body of research to address these gaps in the scientific understanding of sedentary behavior and CVD. Having a greater understanding of these mechanisms could lead to improved treatment and preventative strategies of CVD in public health around the world and in the USA.

The known benefits of exercise for health are very well established and using PA to prevent the negative vascular changes caused from prolonged sitting is a known strategy. Interrupting sitting with moderate intensity PA breaks has been found to be effective for preventing reductions in flow-mediated dilation (FMD), but the effectiveness of intermittent standing interruptions and performing exercise prior to prolonged sitting lack research. This thesis will therefore explore the effectiveness of these strategies on cBP and cfPWV. These findings could provide potential preventative practices and recommendations for governing bodies that aim to reduce CVD and chronic disease risk from desk-job occupations that are very prevalent in the USA.

CHAPTER II – REVIEW OF THE LITERATURE

2.1 Cardiovascular Disease

2.1.1 Background

According to the World Health Organization (WHO) (2016), 17.9 million people die from cardiovascular diseases (CVD) each year around the globe, which accounts for 31% of all deaths worldwide which is the leading cause for mortality. The increases seen in the average life expectancy due to increased economic development and health care management have resulted in superior treatment practices for nutritional deficiencies and infectious diseases (Yusuf et al., 2001). This led to an increased susceptibility to health detriments from the aging process and lifestyle factors that lead to chronic disease development. This shifting is known as 'the epidemiologic transition' and accounts for the increases in the incidence of CVD cases seen since the mid twentieth century (Yusuf et al., 2001). CVD is especially prominent in the USA, with 655,000 deaths per year and costing the country around 219 million dollars each year (CDC, 2020). Prevention of the many variations of CVD is therefore of high importance in public health endeavors.

2.1.2 Terminology and Risk Factors

CVD is characterized by severe disorders to the heart or blood vessels and categorized according to the location of the disorder in the body. Acute CVD most often results from a restriction of blood flow that occurs within a blood vessel (ischemia) that reduces the supply of oxygen and subsequent functioning of its target organ or tissue (American Heart Association [AHA], 2015). Coronary heart disease is the most common

type of CVD and is known as a heart attack, caused by ischemia of the coronary arteries that restricts oxygen supply to the cardiac tissue, quickly leading to death of the heart (AHA, 2015). Cerebrovascular disease is caused by blockage or hemorrhage of the arteries supplying oxygen to the brain, and this can lead to a stroke - brain tissue damage that can cause cognitive or physical dysfunctions (American College of Sports Medicine [ACSM], 2014). Peripheral artery disease refers to ischemia of the arteries serving the arms or legs which leads to pain and sometimes tissue death from a lack of oxygen supply (AHA, 2016). Chronic CVD's can be prolonged conditions of abnormal heart functioning for reasons unrelated to ischemia. Arterial stiffening is not considered a type of CVD but can result from physiological processes related to chronic disease and its significance will be discussed later in this thesis. Factors that put individuals at the highest risk for CVD are clearly presented in clinical settings. The AHA and the ACSM (2018) have identified the risk factors that are most determinant of CVD for health screening purposes based on research. These include age, a family history of CVD, a history of smoking and/or sedentary lifestyle, current hypertension, dyslipidemia, or hyperglycemia, and/or any current disease state of obesity, diabetes mellitus, cardiovascular and renal disease (ACSM, 2016). Other risk factors that are not a part of health screening can include heredity, stress, alcohol use and poor diet or nutrition practices (AHA, 2016). Arterial stiffening is not considered an official risk factor for CVD during health screening, but the possible influences of this vascular condition will be later discussed in this research.

2.2 Arterial Stiffening

2.2.1 Terminology and Causal Mechanisms

Arterial stiffening is the process of reduced distensibility of the arterial vasculature, which reflects a reduced ability to alter the diameter of the arterial lumen to regulate blood flow (Zieman et al., 2005). The medial layers of the arterial wall are essentially composed of a matrix of elastin and collagen fibers that provide a structural integrity to the artery. The inner layers of smooth muscle and endothelial cells serve as mechanisms for controlling blood vessel diameter, with nitrogen oxide release from endothelial cells in response to hemodynamic forces inducing the relaxation of smooth muscle cells and dilation of the blood vessel (Guyenet, 2006). Alterations to the structure or function of these vascular materials due to stable or dynamic processes can lead to reductions in the degree of distensibility in response to increased blood flow. Arteries have been found to become stiffer with age due to increased collagen deposition and the degradation of elastin leading to thicker and less compliant arteries (Zieman et al., 2005). Other metabolic disease states such as hypertension, dyslipidemia, diabetes, obesity, and renal disease have been found to induce matricular hypertrophy and fibrosis of the medial layer of the arterial walls or impairment to NO release from damaged endothelial cells (Benetos et al., 2002; Zieman et al., 2005). This can often result through direct or indirect mechanism that elevate BP and cause prolonged shear stress on the arterial walls (Benetos et al., 2002; Zieman et al., 2005). Since sedentary behavior is positively associated with CVD as well as arterial stiffening itself (Bohn et al., 2017; Patterson et al., 2018), the possible influences of sedentary behavior on arterial stiffening will be discussed later in this thesis.

2.2.2 Associations with Cardiovascular Disease

Arterial stiffness is now being recognized as an epidemic with the main consequence being a reduced regulation of BP during increased blood flow through the vascular network which can lead to hypertension (Mitchell, 2014; Zieman et al., 2005). Hypertension can lead to adverse cardiovascular alterations such as reduced perfusion in the coronary arteries (Zieman et al., 2005), inner ventricular hypertrophy (Vasan et al., 2019), atherosclerosis and vascular embolisms (Cunningham & Gotlieb, 2005). Hypertension from arterial stiffening is therefore a likely critical cause of CVD. Past research would suggest that there could be significant correlations and/or associations between arterial stiffness and cardiovascular disease incidence or development in general populations when measured as PWV. Mattace-Raso et al (2006) found aortic PWV index to be an independent predictor of coronary heart disease and stroke in apparently healthy adults, providing a greater predictive value than CVD risk factors and atherosclerosis. Carotid distensibility was however not independently associated with CVD. Abdullah Said et al (2018) found higher arterial stiffness index and pulse pressure to be independently associated with increased risk of overall CVD, myocardial infarction, coronary heart disease, and heart failure in a general population sample. Vasan et al (2019) found elevated arterial stiffness measures to be associated with the presence of target organ damage such as albuminuria and left ventricular hypertrophy in a general population, which elevated the incidence of CVD by 33%. Other research would suggest that arterial stiffness could be more related to CVD incidence in populations that have risk factors of CVD. Mitchell et al (2010) found higher aortic stiffness assessed using PWV to be associated with a 48% increase in CVD risk in older adults, while

augmentation index, central pulse pressure and pulse pressure amplification were not significantly related. Niiranen et al (2016) found that PWV in non-hypertensive and hypertensive older populations was associated with a trend towards increased CVD incidence. A systematic review and meta-analysis conducted by Vlachopoulos et al (2010) found aortic stiffness measured as aortic PWV to be a strong predictor of future cardiovascular events and all-cause mortality in populations who have more risk factors for CVD at baseline. Risk of CVD was increased by 14% with an increase in PWV of 1 m/s (1 SD) independent of age, sex, and risk factors.

2.2.3 Assessment

Carotid-femoral pulse wave velocity (cfPWV) is the gold standard measure of arterial stiffness. CFPWV measures the stiffness of the aorta and is assessed with the ratio between the time delay between the simultaneously recorded pulse waves in the carotid and femoral arteries and the distance between the carotid and femoral arteries (Mattace-Raso et al., 2006). Higher CFPWV values indicate higher arterial stiffness. Arterial distensibility can be assessed by measuring the displacement of the vessel wall motion and change in lumen diameter of the carotid or femoral artery (Mattace-Raso et al., 2006).

FMD measures the degree of widening of the artery lumen following increased blood flow, while measures of shear stress can also provide an indicator of arterial function during increased blood flow (Thijssen et al., 2011). Although arterial stiffness cannot be directly measured, these assessments of vascular function can provide to be accurate indicators (Segers et al., 2020).

2.3 Physical Activity and Exercise

2.3.1 Background

Physical activity (PA) is defined as any bodily movement produced by skeletal muscle that results in energy expenditure, while exercise is defined as a type of physical activity that consists of planned, structured, and repetitive body movement that is done to improve or maintain one or more components of physical fitness (ACSM, 2016). The absolute intensity of PA can be measured in terms of metabolic equivalents (METs) which is the amount of oxygen consumed relative to body weight per minute. Levels of PA intensity are classified in METs as follows: light (>1.5 to <3), moderate (3 to <6), and vigorous (>6) intensity (ACSM, 2016). Physical activity and exercise have been long found to have a multitude of physiological and psychological health benefits to the human body, which has led to the ACSM in conjunction with other governing bodies to create specific recommendations for physical activity or exercise regarding to the weekly duration and the intensity needed to improve health, and lower morbidity and premature mortality. The US Department of Health and Human Services (DHHS) and the ACSM 2016 latest guidelines recommends that all healthy adult individuals engage in 150-300 minutes of moderate intensity aerobic PA per week, or 75-150 minutes of vigorous intensity aerobic PA per week (or a combination of moderate and vigorous) and perform activities that maintain or enhance muscular strength and endurance at least 2 days per week. Unfortunately, as reported last by the CDC (2018), only 54.2% of adults (aged 18-65 years) in the USA meet these aerobic exercise guidelines, while 27.6% meet the guidelines for muscle strengthening activity, and 24% meet both. This is disappointing

when considering the potential health benefits that can be gained from physical activity for preventing many chronic disease states.

2.3.2 Epidemiology and Health Benefits

As previously stated, the health benefits of PA are evident. Much evidence has demonstrated an inverse relationship between the amount of physical activity or exercise performed (for both aerobic and resistance exercise) and incidence of premature mortality and many disease states including CVD, hypertension, stroke, type 2 diabetes, metabolic syndrome, obesity, and thirteen cancers (Gardner et al., 2011; DHHS, 2018; Nocon et al., 2008). Performing physical activity (especially resistance exercise) throughout the lifespan has also been found to have important health benefits for older age, with inverse relationships being found between PA/exercise and functional health, falls, and osteoporosis (Gardner et al., 2011; DHHS, 2018). This could also potentially reduce sedentary behavior that is typical with older age, and this is important considering the research associating sedentary behavior with mortality and disease incidence, and other research that had reported a 30% reduction in risk of all-cause mortality from sedentary behavior in individuals who are more physically active (Biswas et al., 2015). Furthermore, the inverse relationships between PA and most disease states or health conditions have a dose response relationship, with more PA providing further reductions in disease risk or further health benefits (Baceviciene et al., 2013; Haskell et al., 2007; Kelly et al., 2014; Liu et al., 2017; Smith et al., 2016). This is demonstrated whereby aerobic capacity and muscular strength both have inverse relationships with all-cause mortality, CVD incidence, and risk factors for CVD (Artero et al., 2012; García-Hermoso

et al., 2018; Kodama et al., 2009). The conclusions made from this evidence by the Physical Activity Guidelines Advisory Committee (2018) are that performing moderate amounts of physical activity on most days or all days of the week elicits important health benefits, and individuals who engage in greater amounts or intensities of PA can elicit further health benefits than those who engage in less. Individuals who are physically inactive are classed as sedentary which is a risk factor for CVD (ACSM., 2016).

2.4 Sedentary Behavior

2.4.1 Background

Sedentary behavior is defined by the ACSM (2016) and the Sedentary Behavior Research Network as any waking behavior or activity that is equal to or below 1.5 metabolic equivalents, and examples include sitting and using a computer, watching television, or using light hand tools (Tremblay et al., 2017). Although a sedentary lifestyle or a physically inactive individual has been defined as not participating in at least 30 minutes of moderate intensity physical activity on at least 3 days of the week for at least three months (and is classed as a risk factor for cardiovascular disease), physically active individuals can still exhibit sedentary behavior such as prolonged sitting that has adverse health changes (van der Ploeg & Hillsdon, 2017). Although it if difficult to determine global trends of sedentary behavior, the mean duration of sedentary behavior in the USA for the adult population has been found to be 7.7 hours per day, which equates to 55% of adults'waking day doing activities that involve prolonged sitting (Matthews et al., 2008; WHO, 2020). Sedentary behaviors in recent years have been attributed to the advances in technological innovation, which has increased exposure to

the use of cell phone, television and video devices, made desk job occupations more popular, and increased use of motor vehicle transportation (WHO, 2020), (Fennell et al., 2019). Psychological, sociological and environmental barriers to performing physical activity would also likely play a substantial role in causing sedentary behaviors (Herazo-Beltrán et al., 2017). This is worrying considering that high degrees of sedentary behavior puts individuals at risk for CVD and other chronic disease states (ACSM., 2016).

2.4.2 Epidemiology

It is well established that prolonged sedentary exposures have adverse health consequences. Many studies have found associations between prolonged periods of sitting or sedentary behavior and risk of disease incidence and death, independent of the level of physical activity. This is confirmed by multiple meta-analysis that have found sitting time to be independently associated with a greater risk of all-cause mortality, and incidence of CVD, cancer, and type 2 diabetes mellites in adult populations (Biswas et al., 2015; de Rezende et al., 2014; Patterson et al., 2018). The association between sedentary behavior and hypertension independent of physical activity level would seem apparent. A study conducted by (Lee & Wong, 2015) found BP to be associated with self-reported sedentary time, but not with accelerometer-assessed sedentary time. An additional hour of sedentary behavior per day was associated with increases in systolic BP by 0.06 mmHg and diastolic BP by 0.20 mmHg (Lee & Wong, 2015). Another meta-analysis by Beunza et al (2007) found self-reported total sedentary behavior to be directly associated with higher risk of hypertension. Sedentary behaviors have also been associated with elevated cholesterol levels, with Zhou et al (2017) finding sedentary

behavior to increase the risk of new cases of dyslipidemia in women and increased the risk of prevalent cases of dyslipidemia in men and women.

The associations between acute and chronic sedentary exposure and arterial stiffening seem to be clear. Bohn et al (2017) found sedentary time (measured via accelerometer) to be associated with carotid-femoral pulse wave velocity independent of age, systolic BP, and fasting glucose, while a higher sedentary time in individuals with metabolic syndrome (having at least 3 risk factors for CVD) was associated with worse profiles of arterial stiffness. A meta-analysis conducted by Germano-Soares et al (2018) found a positive relationship between cfPWV and sedentary behavior, indicating longer durations of sedentary behavior are associated with higher arterial stiffness. Furthermore, arterial stiffening may be more prone in the central and lower extremity vasculature. A meta-analysis by Paterson et al (2020) found that FMD was significantly decreased in the lower but not upper limbs from prolonged sitting, while Credeur et al (2019) found significant increases in aortic pulse waive velocity from 3 hours of prolonged sitting. It therefore seems likely that sedentary behavior can induce arterial stiffening acutely through vascular mechanisms or chronically through metabolic mechanisms.

2.4.3 Mechanisms Linking Sedentary Behavior to Adverse Vascular Alterations

Although the associations of sedentary behavior with mortality, various disease incidence, and other adverse health consequences is very clear, the exact physiological mechanisms that cause these changes in the human body is not currently known (Park et al., 2020). There are however many proposed hypotheses which can give possible explanations and an understanding for how sedentary behavior leads to chronic adverse

vasculature changes (Park et al., 2020). A lack of shear stress on the vasculature from sedentary exposure would seem to be a likely mechanism. A study by Demiot et al. (2007) found impaired endothelial function in the microvasculature from sedentary exposure. Measures of endothelium dependent and independent vasodilation and levels of circulating endothelial cells (CEC's) (index of endothelial damage) were compared between two groups of 8 women who all underwent 2 months of bed rest; an exercise group performing treadmill and resistance exercise, and a control group that performed no exercise. The results found the control group had significantly reduced endothelium-dependent vasodilation and significantly increased CECs from baseline, while there were no changes in the exercise group. These vascular changes were proposed to result from the chronic reductions in blood flow from physical inactivity and low metabolic demands, which places minimal shear stress on the endothelial linings of the vessels that leads to endothelial cell dysfunction and apoptosis (CEC's) (Demiot et al., 2007). Furthermore, studies have demonstrated endothelial dysfunction in the popliteal and femoral arteries of the legs after acute exposure to prolonged sitting, and this is also thought to be caused from decreased blood flow-induced anterograde shear stress (Restaino et al., 2015, 2016; Thosar et al., 2015). Although the exact mechanisms that link reduced shear stress to endothelial dysfunction are not known, it is thought that the production of enothelin-1 from a lack of shear stress (which is responsible for increasing vascular reactive oxygen species and reducing nitric oxide bioavailability) is a possible mechanism (Padilla & Fadel, 2017). These impairments of vascular function from acute and chronic sedentary exposure are however likely be possible mechanisms for the development of hypertension, arterial stiffness, and CVD from sedentary behavior.

Other research suggests that reductions in lipoprotein lipase (LPL) activity from sedentary exposure may play a role. A study by Yanagibori et al (1998) found twenty days of bed rest significantly increased very low-density lipoprotein cholesterol (LDL) and fasting plasma insulin levels, while significant decreases were found in LPL activity, high-density lipoprotein cholesterol levels (HDL), and insulin sensitivity. LPL is a protein that plays a role in transporting fat molecules to necessary cells and tissues of the body, and the diminished LPL activity would have likely resulted in the low concentrations of HDL's and high concentrations of LDL's which are both risk factors for dyslipidemia, atherosclerosis, and CVD development (Yanagibori et al., 1998). Metabolic risk markers from other chronic disease states may also play a role. The declines in insulin sensitivity and increases in lipid deposition found from sedentary exposures through muscle inactivity and positive energy balances can also lead to the development of metabolic syndromes, such as hypertension, type 2 diabetes and obesity which are risk factors for CVD, with coinciding pathophysiological mechanisms that are more well understood (Dempsey et al., 2018). Glucose intolerance and insulin resistance have also been linked with the glycation of vascular wall proteins and the cross linking of collagen in the arterial walls, causing subsequent increases in arterial stiffness (Zieman et al., 2005).

2.5 Intervention Strategies

2.5.1 Background

With prolonged sitting being unavoidable in the workplace and other environments, much research has focused on whether breaking or interrupting prolonged

sitting with PA/exercise or replacing sitting time with standing provides health benefits or reduces adverse health consequences of prolonged sedentary exposures. Performing exercise prior to sedentary exposure has also been a devised strategy. Many studies have looked at the acute effects on cardiometabolic markers such as postprandial glucose, insulin, and triglyceride levels, which could chronically increase the risk of cardiometabolic disease by inducing oxidative stress, inflammation, and endothelial dysfunction in vascular structures over time (Loader et al., 2015; O'Keefe & Bell, 2007). Thorough research has found interrupting prolonged sitting with moderate intensity PA breaks to be an effective strategy for reducing these metabolic markers (Loh et al., 2020; Quan et al., 2021; Saunders et al., 2018). Other studies have examined the acute and chronic effects of these interventions on vascular function be measuring variables of BP and flow-mediated dilation. These variables can provide indications of acute arterial stiffening from prolonged sitting that may lead to CVD, as found in the literature (Paterson et al., 2020).

2.5.2 Interrupting Prolonged Sitting with PA Breaks

Interrupting strategies would seem to be effective for offsetting adverse vascular changes in healthy adults. A meta-analysis by Paterson et al (2020) found that flow-mediated dilation was significantly higher with interrupted prolonged sitting compared to uninterrupted prolonged sitting in healthy populations. Subgroup analysis found aerobic and simple resistance activities to have moderate non-significant effects for flow-mediated dilation, while the one study that tested standing interruptions only had trivial effects (Paterson et al., 2020).

Fewer studies have examined the effects of PA breaks on BP. Dempsey et al (2018); Larsen et al (2014); Zeigler et al (2016) found BP to be higher during prolonged sitting compared to with intermittent PA breaks in adults who were either overweight, obese, or diabetic while Wennberg et al (2016) did not find any differences. Bailey & Locke (2015) also found no differences in healthy individuals.

The current evidence would suggest interrupting prolonged sitting with intermittent PA breaks of moderate intensity can elicit benefits to flow mediated dilation while more research is needed for the effects of intermittent standing breaks. Although PA breaks may pose benefit for inducing positive BP changes, more research is needed on heathy populations.

2.5.3 Replacing Prolonged Sitting Time with Standing

Longitudinal studies have examined the long-term health benefits of replacing sitting with standing in the workplace, usually through standing or stand-sit workstations. A meta-analysis conducted by Saeidifard et al (2020) found replacing sitting with standing for an average of 1.33 hours per day for 3.81 months was not effective for eliciting changes in BP. Although it was specified that potential health benefits from standing may differ in individuals depending on baseline PA level, body composition, age and sex, more research is required to determine these differences (Saeidifard et al., 2020). A meta-analysis by Paterson et al (2020) also concluded that replacing sitting with standing may pose more benefit to vascular function than intermittent standing breaks, but more research is needed to determine this. So et al (2018) also found replacing 1 hour of sitting time with standing or walking in the workplace was associated with a 7%

reduced risk of heart disease. More research is generally needed to determine any long-term benefits to vascular function benefits by replacing prolonged sitting with standing.

2.5.4 Performing Exercise Prior to Prolonged Sitting Bouts

The vascular health benefits of acute PA seem to be apparent, with increases in vascular function and lowered BP that last for hours after the cessation of exercise (Carpio-Rivera et al., 2016; Green & Smith, 2018). However, whether adverse vascular changes from prolonged sitting can be reduced with the intervention strategy of performing PA or exercise prior to prolonged sitting has not been directly investigated, with limited experimentally controlled research being conducted for this approach. Ballard et al (2017) found femoral artery flow-mediated dilation declined from baseline at 1, 2, and 3 hours of prolonged sitting, and resting shear rate declined at 3 hours, and performing 45-minute bouts of continuous treadmill exercise at 65% of the participants maximum oxygen consumption (VO2 max) prior to sitting was effective for preventing these reductions in healthy men, independent of changes in oxidative stress, endothelin-1, and nitric oxide status in the vasculature. Morishima et al (2017) also found that a prior 45-minute bout of cycling exercise (at 50-87% of HR max) prevented the significant popliteal artery flow-mediated dilation impairment that occurred from prolonged sitting without prior exercise in healthy adults. Although more research is needed for this intervention strategy, it would seem likely that performing prior exercise could elicit positive vascular changes in both healthy and unhealthy populations. This could be especially important for those who use physical activity such as walking or cycling to commute to occupations which involve prolonged sitting.

2.6 Summary

The current epidemiological research regarding the development of CVD from chronic sedentary behavior are well recognized, but the current evidence for the physiological mechanisms that link chronic sedentary behaviors to CVD development need further review for which this thesis will address. The health benefits of regular PA are also well established, as well as the effectiveness of certain PA strategies for offsetting adverse health changes from prolonged sitting. But more research is needed on the strategies of intermittent standing breaks and performing prior exercise for preventing negative vascular changes, which presents gaps in the research for which this thesis will explore. The acute vascular changes that result from exposures of sitting might be linked to CVD development and need further investigation, and whether standing and performing prior exercise are effective strategies for reducing arterial stiffening from prolonged sitting remains yet to be determined.

This thesis will add to the growing body of research that addresses the vascular health changes that occur from prolonged sitting exposure, and this will help further the scientific understanding for the physiological mechanisms that link sedentary behavior to CVD – the leading cause of death in the USA. Furthermore, this thesis will provide additional research concerning to PA strategies for reducing adverse vascular changes from prolonged sitting, which will be useful for the many individuals who have desk job occupations. Fields and departments within public health will therefore benefit from the current thesis study, by having the potential for a greater understanding of how CVD results from sedentary exposure and a greater knowledge for what can be done to resolve the issues of sedentary exposure more effectively.

CHAPTER III – METHODOLOGY

3.1 Participants

Fifteen participants (healthy males and females aged 18-45 years of any ethnicity) were recruited from The University of Southern Mississippi (USM) and the surrounding Hattiesburg area to participate in the study. Although one study has found females to not exhibit significant aortic stiffening from prolonged (Creduer et al., 2019), individual vascular responses to prolonged sitting have not been found to significantly differ between genders in other literature (Vranish et al., 2017; O'Brien et al., 2019). Fluctuating hormones due to the menstrual cycle potentially influence vascular responses to prolonged sitting, but only one study by O'Brien et al (2020) has explored this directly. Their findings found negative lower limb vascular effects which did not significantly differ across phases of the menstrual cycle (O'Brien et al., 2020). Peripheral vascular function has been found to possibly elevate during the follicular phase but with very little certainty (Williams et al., 2020), while central vascular function has not been found to vary across phases of the menstrual cycle but with very low certainty also (Williams et al., 2020; Ounis-Skali et al 2006; Skali et al 2002; Priest et al 2018). Although this evidence would not suggest there is good reason to control for gender or menstrual cycle phase during prolonged sitting, the present study chose to limit participation to males only due to the nature of the measurement techniques being unfeasible with the principal investigator.

Differences due to physical activity level are largely unexplored, although one study by Creduer et al (2019) found no influence of physical activity status on vascular

function due to prolonged sitting, and adverse vascular changes from prolonged sitting are still very apparent in healthy individuals (Paterson et al., 2020; Saunders et al., 2018). However, to control for possible differences due to health and fitness level, elite athletes, individuals with current or past known cardiovascular, metabolic, or neurological disease, on medication that affects hemodynamics, or current/past smokers (quit within last six months) were excluded from the study. Age has been shown to have significant effects on vascular stiffness and is a risk factor for CVD at 55 years or older for males and 65 years or older for females (ACSM., 2016; Zieman et al., 2005). The inclusion criteria of 45 years therefore provide a leeway of 10 years on any vascular differences that may exist due to age. Individuals who were contraindicated from moderate intensity exercise (due to pregnancy, musculoskeletal injury, back pain) due to ACSM (2016) guidelines were also excluded from the study. Table 3.1 below shows the inclusion and exclusion criteria for the present study.

Table 3.1 *Inclusion and Exclusion Criteria*

Inclusion Criteria: • Male • Aged 18-45 years • Able to attend 4 separate sessions of 3.5- to 4-hour duration
Exclusion criteria: • Current or previous cardiovascular, metabolic, and or neurological disease • Currently taking medication that affects cardiovascular measures (ie., beta blockers, ACE inhibiters, Metformin, etc) • Current smoker or cessation of smoking within last 6 months • Current pregnancy, back pain or musculoskeletal injury that contraindicates moderate intensity exercise • Current elite athlete

3.2 Recruiting and Screening Procedures

Participants were recruited through word of mouth and email invitations that were sent to individuals such as USM students via The School of Kinesiology and Nutrition department (see Appendix C), and flyers (see Appendix B), including to the surrounding Hattiesburg area. A questionnaire that addressed inclusion and exclusion criteria was administered through surveymonkey.com via the flyer to interested candidates (see Appendix D) to ensure they met the criteria to participate in the study. This included their perceived ability to attend the sessions and their previous and current medical history. If candidates were found to be eligible to participate, they specified four different test days via email that they were available to attend the lab to participate in the study.

3.3 Experimental Design

The present study was a randomized-crossover design, whereby participants reported to four separate and experimental sessions of around 3.5- to 4-hours in duration. The sessions were conducted within an exercise physiology research laboratory that was a simulated office space within The School of Kinesiology and Nutrition.

Upon arrival at the laboratory, an informed consent form was provided to the participants that was read vocally by the investigator to the participant. This included the purpose of the study, the benefits and risks of participation and the participants' right to withdraw at any time. Any questions from the participant were answered at that time. If the participant agreed to participate, they signed the form by writing their full name and signature in accordance with the USM Institutional Review Board (IRB). After written

consent to participate had been obtained, participants will complete a PAR-Q+ health history form to ensure it was safe for them to perform physical activity, as recommended by the ACSM. Participants who were found to require medical clearance to perform physical activity based on their answers to the PAR-Q+ form were immediately excluded from the study.

3.4 Experimental Measures

The present study included the following experimental measurements; anthropometrics (height, weight, BMI), brachial and central systolic and diastolic BP, carotid-femoral pulse wave velocity (cfPWV) and resting heart rate. Anthropometric measures were assessed at the start of every visit. Brachial and central blood BP was assessed at baseline, following every hour of the intervention (at 1-hour and 2-hours) and post completion of the intervention for each condition. CfPWV was assessed at baseline and post completion of the intervention for each condition. Resting heart rate was assessed before the start of any interventions that contained exercise. The time points for each measurement are shown in **Table 3.2** below.

Table 3.2 *Timeline of Assessments*

Measurement	Pre-Intervention	Intervention		Post-Intervention
		1 hours	2 hours	
Anthropometrics	*X*			
Brachial Blood Pressure	*X*	*X*	*X*	*X*
Central Blood Pressure	*X*	*X*	*X*	*X*
Carotid-Femoral PWV	*X*			*X*
Resting HR (exercise visits only)	*X*			

3.4.2 Anthropometrics

Measures of height and bodyweight were assessed after the written informed consent and PAR-Q+ were completed on the first visit and assessed at the start of the participants' future visits. The participants were asked to remove their shoes and any other excessively heavy clothing or loose objects from their pockets. Participant's height was assessed using a wooden stadiometer to the nearest 0.25 centimeter (cm) and the participants bodyweight was assessed by using a digital scale to the nearest 0.1 kilogram (kg). These values were used to assess the participants body mass index (BMI) which is calculated and expressed as kg/m^2.

3.4.3 Brachial and Central Blood Pressure

Brachial and central BP were assessed pre- and post-intervention and following each hour of the intervention (at 1-hours- and 2 hours- of intervention completion) via automated sphygmomanometry using the method of oscillometric assessment (SphygoCor XCEL, AtCor Medical, Itasca, Illinois). Two measurements were made at each time of assessment and averaged, and a third measurement was made if the two values were outside 10 mmHg from another, with the closest two measurements being averaged. Brachial and central BP were assessed by using an XCEL arm cuff of appropriate size that measured brachial BP in the participants right arm, and a brachial pressure waveform determined a sub-systolic inflation to measure central BP. The oscillometric function on the device is a validated method for analyzing brachial waveforms (Butlin et al., 2012).

3.4.4 Carotid-Femoral Pulse Wave Velocity

Aortic vascular stiffness was assessed by using measures of cfPWV pre- and post-intervention via applanation tonometry (SphygmoCor XCEL, AtCor Medical, Itasca, Illinois). Two measurements were conducted at each time of assessment and averaged. Participants started by lying in a supine position on the lab table. A femoral BP cuff was wrapped firmly and as high as possible around the participants right upper thigh. The distance from the participants strongest identified carotid pulse site to their sternal notch, and their sternal notch to the proximal edge of the femoral cuff was measured using a Gulick tape measure. Carotid-femoral transit time (Δt) was then obtained by conducting applanation tonometry over the right common carotid artery. This was paired with oscillometry by using the femoral cuff that is placed over the femoral artery. The difference between the two distance measures divided by the carotid-femoral transit time was calculated through the device to find the cfPWV (m/s). CfPWV has been previously validated for measuring arterial stiffness (Butlin et al., 2013).

3.4.5 Resting Heart Rate

Resting heart rate was assessed before any interventions that involved performing exercise. After pre-intervention assessments of BP and aortic stiffness were made, participants were instructed to put on a polar heart rate monitor at the level of their xiphoid process while sitting on the lab bench. The reading on the heart rate monitor was recorded at two different time points separated by one minute while the participant was sitting. These two recordings were averaged, unless they were outside a 5 beats/minute

range from one another, in which case a third measurement was taken, and the closest two values were averaged.

3.4.6 Questionnaire

Participants were given a physical activity questionnaire called the Godin Leisure-Time Exercise Questionnaire (Appendix I) to assess an approximate amount of exercise they typically perform each week. The questionnaire could be completed at any time during the sitting intervention.

3.5 Experimental Session

All participants will report to the Exercise Physiology Research laboratory on four separate occasions to complete the four experimental conditions of the study, performing one condition on each visit. The conditions will be as follows; 1) uninterrupted sitting only (control condition 'SO'), 2) sitting plus intermittent standing (condition 'SSt'), 3) prior exercise plus uninterrupted sitting (condition 'ES'), 4) prior exercise plus sitting plus intermittent standing (condition 'ESSt'). Participants completed the control condition first, then followed by the strategy conditions which were randomized by using a random number generator with numbers of 2-4 that corresponded to each condition, respectively. The approximate start time of each session was in the morning at 7-8 am or in the afternoon at 1pm, which mimicked a regular work or study day, and each visit was a minimum of 210-minutes and a maximum of 255-minutes in duration. To avoid the fluctuations in BP that may occur throughout the day that could induce variations in the vascular responses to prolonged sitting and exercise (Jones et al.,

2008), participants were required to complete all four of their visits during the same time of day, either all morning or all afternoon sessions. Participants were asked to eat a minimum of 2-hours before the start of each session to avoid having postprandial hypertension during assessment. Participants were asked to abstain from moderate to vigorous exercise in the 24 hours prior to any visit, and abstain from caffeine, nicotine, and alcohol consumption in the 12 hours prior to each visit. In addition, if a participant completes a visit that has an exercise intervention, then they will be required to abstain from future visits on the day that follows due to the potential acute health changes gained from the exercise intervention that may affect vascular measures on the following day. Participants confirmed their adherence to these precautions via word of mouth and were asked to use the rest room prior to the start of each session.

For the first visit, after the participants informed consent and anthropometric measures had been obtained, participants were asked to lie down on the examination table in a supine position. For their future visits, this was done immediately after the anthropometric measures had been obtained. After participants had rested quietly for 10-minutes, their baseline measures of brachial and central BP, cfPWV, and resting heart rate (if performing an exercise intervention) were assessed. Participants then performed their randomly assigned intervention from the four conditions outlined in **Table 3.3** and described in detail below.

Table 3.3 *Summary of Experimental Conditions*

Condition	3 Hours of Sitting	Intermittent Standing of 10-min every 30-min	30-min of Moderate Intensity Exercise
Sitting Only	*X*		
Sitting + Standing	*X*	*X*	
Prior Exercise + Sitting	*X*		*X*
Prior Exercise + Sitting + Standing	*X*	*X*	*X*

After performing their assigned intervention, participants were once again asked to quietly lie in a supine position on the examination table. After 10-minutes, measures of brachial and central BP and cfPWV were assessed. Following these measures, the participant agreed on a time for their next visit before they left the research lab.

3.5.2 Sitting Only Condition

Participants will be asked to sit at a sit-stand work desk next to the examination table for a 3-hour duration. The participants were allowed to study, complete schoolwork on their laptop, or be productive by other means. They however were not allowed to stand up at any time. The investigator did not have to be present for the entire duration of the intervention but visited the room on occasion to check that the participant was following procedures correctly. Following every hour of the intervention (at 1-hours and 2-hours), the participants were instructed to outstretch and rest their arm on the table while they were sitting to repeat the brachial and central BP measurements.

3.5.3 Sitting Plus Intermittent Standing Condition

Participants were asked to sit at a sit-to-stand work desk next to the examination table for a 3-hour duration and were asked to stand for 10-minutes every 30-minutes (see **Figure 3.1** below). Participants were familiarized with how to use a sit-stand work desk correctly before undergoing this intervention strategy, with the main cues being to avoid leaning on the desk with the arms, leaning excessively on one foot, or crossing the legs at any time. Participants could however naturally alter their stance during the intervention as they typically would in an office setting, if their stance didn't become inappropriate as deemed above. The investigator was present to vocally tell the participant when to stand up and when to sit down and to ensure the participant followed standing procedures correctly. Procedures during sitting and during measurement were the same as described above.

3.5.4 Prior Exercise Plus Sitting Condition

Participants performed moderate intensity exercise at 40-60% of their VO2 max for 30-minutes. Participants were instructed to straddle the motorized treadmill (Trackmaster, USA) and to engage on the treadmill when ready. A 2-minute warm-up was conducted at a slow and comfortable walking speed for the participant. The treadmill speed was increased to a reasonable moderate intensity speed for the participant, and adjusted accordingly so that the participant's heart rate fell within 40-60% of their target heart rate. When 40-60% of their target heart rate was achieved, the 30-minute timer was started, and the treadmill speed was adjusted accordingly and kept as close to 50% of the participants target heart rate throughout the duration of exercise. Participants target heart

rate was based on their heart rate reserve (HRR) value that considers their resting heart rate and age. Participants target heart rate was calculated by using the equation as follows: *Target Heart Rate (beats/min) = (220 – age – HR_{rest}) * Desired % + HR_{rest}*

This allowed the exercise intensity to be the same between individuals by considering individual factors of aging and aerobic fitness level that had potential to affect the participants physical exertion during exercise. After the 30 minutes of exercise was complete, participants completed the procedures for the Sitting Only condition as described above.

3.5.5 Prior Exercise Plus Sitting Plus Intermittent Standing Condition

Participants firstly performed the procedures for moderate intensity exercise exactly as described above. They will then follow the procedures for the Sitting Plus Intermittent Standing condition as described above.

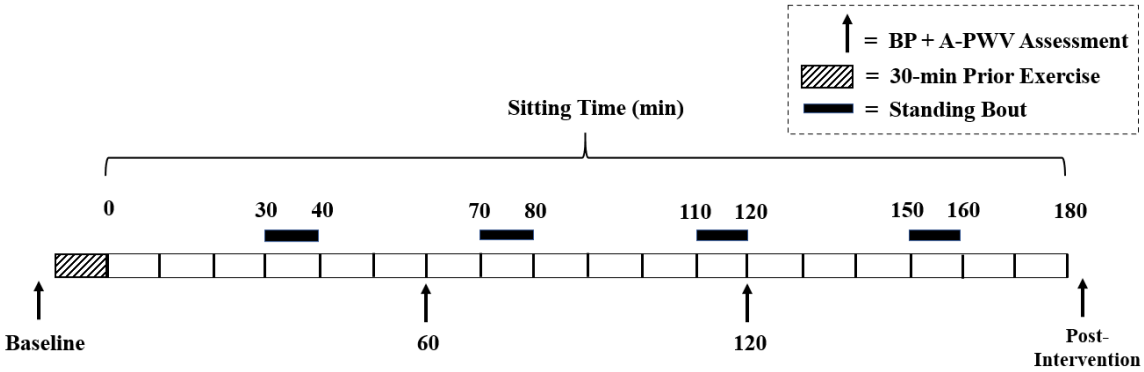


Figure 3.1 *Intervention Design (standing bouts and prior exercise when applicable)*

3.6 Statistical Analysis

Data was collected via a Microsoft Excel spreadsheet and statistically analyzed using IBM SPSS software. Statistical significance was set at $p \leq 0.05$. Necessary steps were taken to normalize the data (i.e., nonparametric tests and log transformations).

Descriptive characteristics were calculated including age, height, BMI, and sedentary behaviors from the physical activity questionnaire.

Participants brachial and central BP and cfPWV responses due to time and condition were analyzed by using multiple two-way repeated measures ANOVA's. Depending on whether statistical interactions were found, changes in brachial and central BP and cfPWV due to time or condition were explored individually, with post-hoc comparisons comparing specific conditions and time points separately.

CHAPTER IV – RESULTS

The purpose of this study was to determine the effectiveness of intermittent standing, prior exercise, and a combination of both as strategies for mitigating the negative vascular response to prolonged sitting. This study utilized a randomized-crossover design to measure vascular health responses in participants which were repeated over four separate conditions. This established the primary aim of making within- and between-group comparisons. All procedures for the study were conducted in a simulated office space located in the School of Kinesiology and Nutrition at the University of Southern Mississippi.

4.1 Participants

Fifteen (N=15) adult males between the ages of 18-45 participated and completed the entirety of the study protocol. The participants' average age was 22.7 ± 3.7 years, with an average height of 1.77 ± 0.073 meters, weight of 87.5 ± 24.0 kg, and BMI of 27.8 ± 7.3 kg/m^2. Demographic characteristics are displayed in Table 4.1.

Table 4.1 *Participant Demographics*

	Average	Range	S.D.
Age (years)	22.7	13.0 (18.0 – 31.0)	3.7
Height (m)	1.77	0.25 (1.67 - 1.92)	0.07
Weight (kg)	87.5	86.7 (60.5 - 147.2)	24.0
BMI (kg/m^2)	27.8	24.1 (20.0 - 44.1)	7.3

Participant results from the Godin Leisure-Time Exercise Questionnaire are reported in Table 4.2. The average number of exercise bouts per week during their free

times were the following: 5.9 ± 4.2 mild bouts/week, 3.2 ± 1.9 moderate bouts/week, 2.3 ± 1.5 strenuous bouts/week. Participants' average physical activity score per week was 54.8 ± 21.5 units, and their frequency of bouts per week that were 'long enough to work up a sweat' was reported as the following; rarely (1), sometimes (7), often (7).

Table 4.2 *Participant Physical Activity Level*

	Average	Range	S.D.
Mild (bouts/week)	5.9	13.0 (1.0 – 14.0)	4.2
Moderate (bouts/week)	3.2	6.0 (0.0 – 6.0)	1.9
Strenuous (bouts/week)	2.3	4.0 (0.0 – 4.0)	1.5
Overall Score (no units)	54.8	73.0 (26.0 – 99.0)	21.5
Freq Sweat Bouts		Rarely (1), Sometimes (7), Often (7)	

4.2 Analysis By Specific Aim

4.2.1 Specific Aim 1: To determine sitting-induced vascular responses with time depending on the utilization of intermittent standing, prior exercise, or combination strategies

The purpose of this analysis was to determine how the changes in the vascular parameters of brachial systolic BP, brachial diastolic BP, central systolic BP, central diastolic BP, and cfPWV due to prolonged sitting are influenced depending on the intervention strategies utilized and the duration of each intervention. Intervention strategies included Sitting Plus Standing, Exercise Plus Sitting, and Exercise Plus Sitting Plus Standing, with the addition of a control condition Sitting Only, each with time points; pre-intervention, 1-hour-intervention, 2-hours-intervention, and post-intervention.

4.2.1.1 Carotid-Femoral PWV – Condition-Time Interaction

A two-way repeated measures ANOVA was performed to determine the effect of the intervention strategies over time on central-femoral PWV. Outliers were determined for each condition separately via the method of inspection of values outside 1.5 box-lengths from the edge of the box in a boxplot (Smiti., 2020). One outlier was detected within the Sitting Only condition, and three outliers were detected within the Intermittent Standing condition, one of which being outside 3-box lengths and classed as an extreme outlier. Upon inspection of outliers, the outlier during Sitting Only (participant ID=2) was determined to be caused by measurement error due to inter-tester variability, as noted during the experimental protocol, and was omitted from the analysis. This outlier was found to have significant impact on the data when sensitivity testing was conducted with the outlier included. The other outliers could not be derived to stem from causes outside of individual physiology and were kept in the analysis. The differences in central-femoral PWV between pre-intervention and post-intervention were normally distributed for all conditions ($p>0.05$) as assessed using Shapiro-Wilk's test for normality. Mauchly's test of sphericity indicated that the assumption of sphericity was met for the two-way condition-time interaction ($\chi^2(2)=8.382$, $p=0.138$). Condition-time interactions on cfPWV are displayed in Figure 4.1.

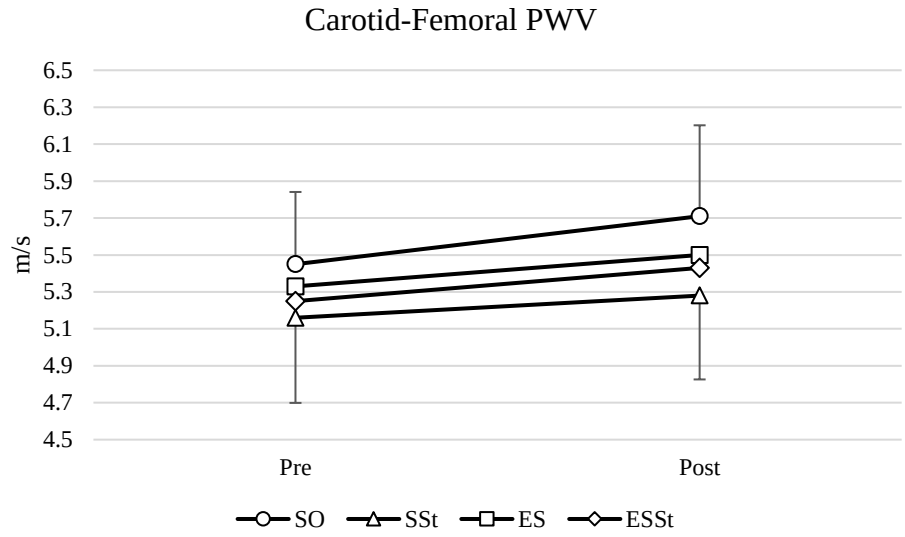


Figure 4.1 *Carotid-Femoral PWV – Condition-Time Interactions*

There was no statistically significant two-way interaction between condition and time for central-femoral PWV ($F(3,39)=1.078$, $p=0.369$). Therefore, main effects were later run individually for condition and time.

4.2.1.2 Blood Pressure – Condition-Time Interaction

A two-way repeated measures ANOVA was conducted to determine the effects of different intervention treatments over time on brachial systolic BP, brachial diastolic BP, central systolic BP, and central diastolic BP. Analysis of studentized residuals found no outliers, as assessed by examination of values greater than ± 3 standard deviations. All BP variables were normally distributed ($p>0.05$) except for brachial systolic at 2-hours-intervention of the sitting plus standing condition and central systolic BP at 2-hours-intervention of the exercise plus sitting condition, as assessed using Shapiro-Wilk's test for normality. Mauchly's test of sphericity indicated that the assumption of sphericity

was met for the two-way treatment-time interaction for brachial systolic BP ($\chi^2(2)=59.05$, $p=0.094$) and central systolic BP ($\chi^2(2)=61.64$, $p=0.062$), but was violated for the two-way interaction for brachial diastolic BP ($\chi^2(2)=64.595$, $p=0.038$) and central diastolic BP ($\chi^2(2)=73.24$, $p=0.007$). Condition-time interactions on BP parameters are displayed graphically in Figures 4.2, 4.3, 4.4, 4.5, 4.6 and 4.7. There was no statistically significant two-way interaction between treatments and time for any BP variables. Therefore, main effects were later run individually for condition and time.

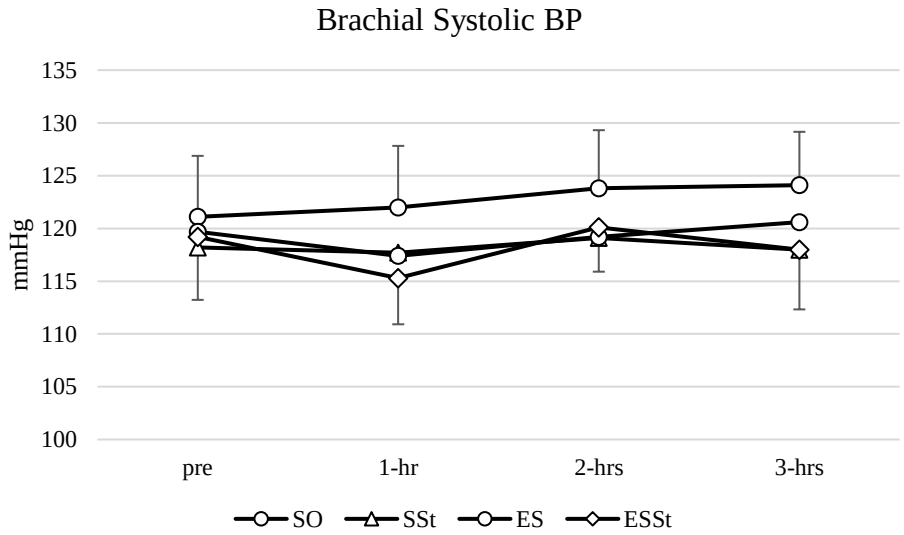


Figure 4.2 *Brachial Systolic BP – Condition-Time Interactions*

There was no statistically significant two-way interaction between conditions and time for brachial systolic BP, $F(9, 126)=0.822$, $p=0.597$.

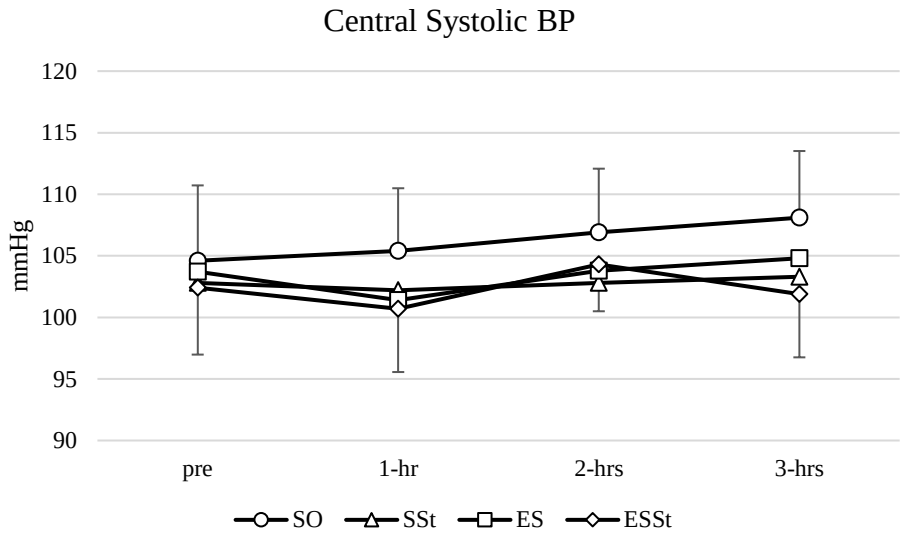


Figure 4.3 *Central Systolic BP – Condition-Time Interactions*

There was no statistically significant two-way interaction between conditions and time for central systolic BP, (F(9, 126)=0.799, p=0.618).

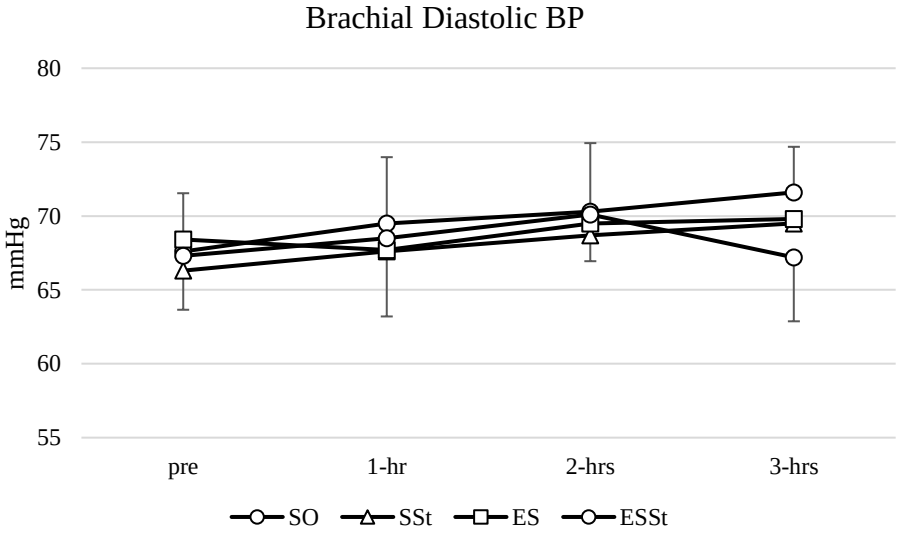


Figure 4.4 *Brachial Diastolic BP – Condition-Time Interactions*

There was no statistically significant two-way interaction between conditions and time for brachial diastolic BP, (F(4.6, 64.3)=0.925, p=0.465).

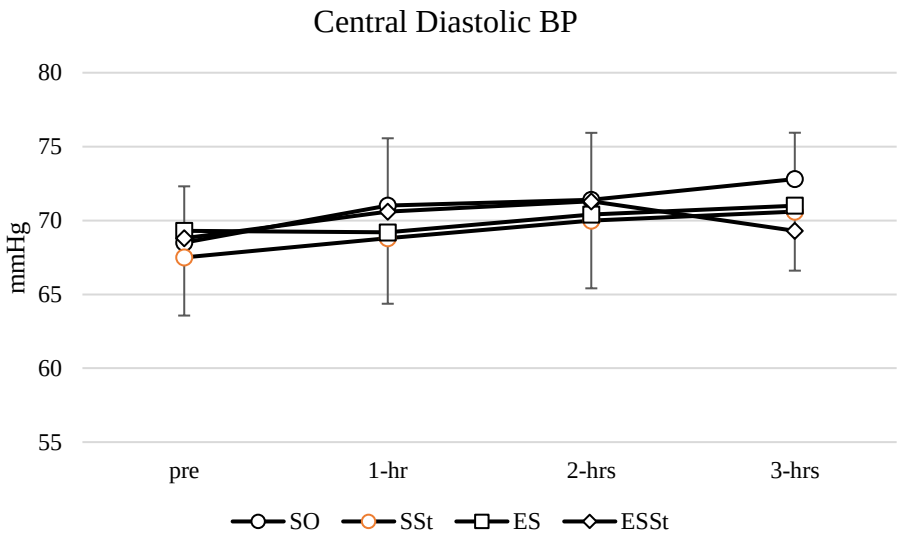


Figure 4.5 *Central Diastolic BP – Condition-Time Interactions*

There was no statistically significant two-way interaction between conditions and time for central diastolic BP, (F(4.7, 65.7)=0.785, p=0.557).

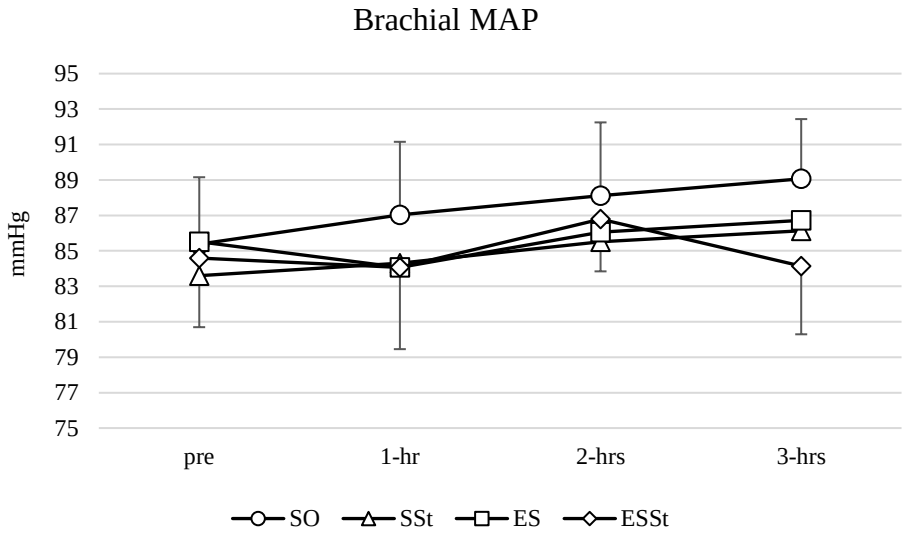


Figure 4.6 *Brachial Mean Arterial Pressure – Condition-Time Interactions*

There was no statistically significant two-way interaction between conditions and time for brachial MAP, (F(9.0, 13.34)=1.116, p=0.356).

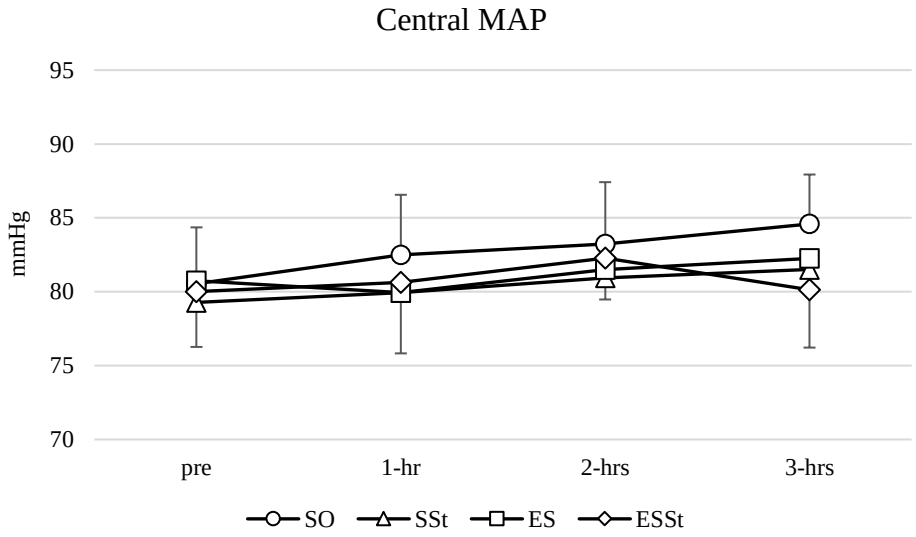


Figure 4.7 *Central Mean Arterial Pressure – Condition-Time Interactions*

There was no statistically significant two-way interaction between conditions and time for central MAP, (F(9.0, 11.12)=0.893, p=0.534).

4.2.2 Specific Aim 2: To determine how sitting-induced vascular health responses differ between the strategies of intermittent standing and/or prior exercise

The purpose of this analysis was to determine how the vascular parameters of cfPWV, brachial systolic BP, brachial diastolic BP, central systolic BP and central diastolic BP compared between the intervention strategies utilized, at time points of pre-intervention, 1-hr-intervention, 2-hours-intervention, and post-intervention.

4.2.2.1 Carotid-Femoral PWV – Condition Individual Main Effects

Individual main effects of condition on cfPWV for each time point (pre- and post-intervention) were analyzed using a one-way repeated measures ANOVA. The assumption of sphericity was met for the main effects of condition for each time point. Condition individual main effects on cfPWV for each time point are displayed graphically in Figure 4.8.

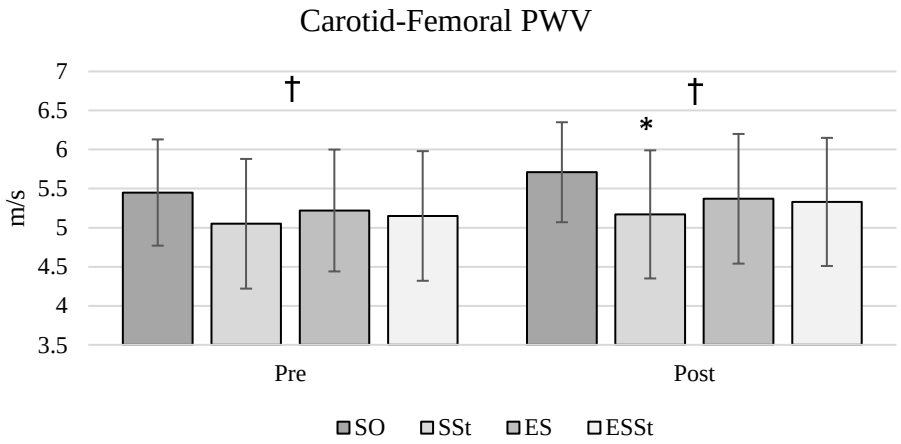


Figure 4.8 *Carotid-Femoral PWV – Condition Main Effects*

†Denotes significant individual main effects of condition at specific time point ($p<0.05$)

*Denotes significant difference from condition SO ($p<0.05$)

Individual main effects of condition showed a statistically significant difference in cfPWV between conditions at pre-intervention ($F(3, 39)=3.147$, $p=0.036$) and post-intervention ($F(3, 39)=6.637$, $p<0.001$). Therefore, Sidak adjusted post-hoc pairwise comparisons were run. Data are mean ± standard error, unless otherwise stated. At post-intervention, central-femoral PWV was significantly higher during the Sitting Only condition (5.714 ± 0.172 m/s) compared to the Intermittent Standing condition (5.275 ± 0.195 m/s), a significant difference of 0.439 (95% CI, 0.153 to 0.726) m/s, p=0.002.

4.2.2.2 Blood Pressure – Condition Individual Main Effects

Individual main effects of condition on each BP variable for each time point were analyzed using a one-way repeated measures ANOVA. The assumption of sphericity was met for main effects of condition on all BP variables for all time points, except for brachial diastolic BP at 1-hr-intervention ($\chi^2(2)=14.922$, $p=0.011$) and central diastolic BP at 1-hr-intervention ($\chi^2(2)=14.144$, p=0.015). Condition individual main effects on cfPWV are displayed graphically in Figures 4.9, 4.10, 4.11, 4.12, 4.13, 4.14.

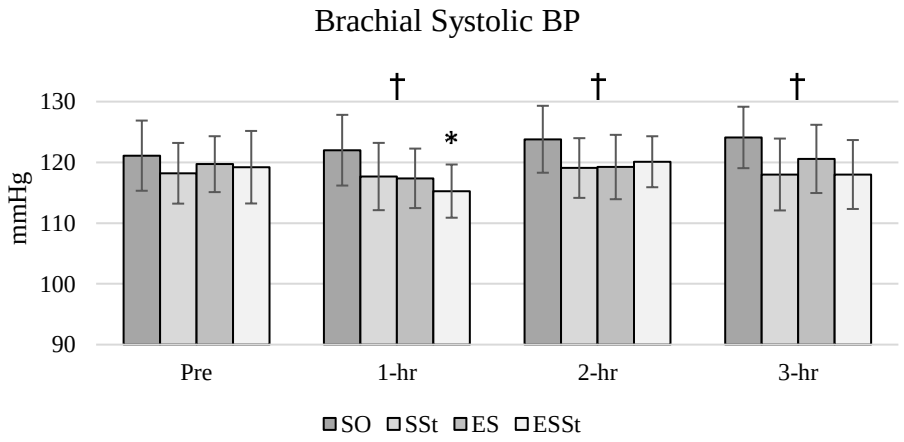


Figure 4.9 *Brachial Systolic BP – Condition Main Effects*

†Denotes significant individual main effects of condition at specific time point (p<0.05)

*Denotes significant difference from condition SO (p<0.05)

Individual main effects of condition showed a statistically significant difference in brachial systolic BP at 1-hour-intervention ($F(3, 42)=6.427$, $p=0.001$), 2-hours-intervention ($F(3, 42)=3.359$, $p=0.028$), and 3-hours-intervention ($F(3, 42)=3.256$, $p=0.031$). Therefore, Sidak adjusted post-hoc planned pairwise comparisons were run. Data are mean ± standard error, unless otherwise stated. At 1-hr-intervention, brachial systolic BP was significantly higher during the Sitting Only condition (122.033 ± 2.714

mmHg) compared to the Exercise Plus Sitting Plus Standing condition (115.267 ± 2.043 mmHg), a significant difference of 6.767 (95% CI, 1.932 to 11.602) mmHg, p=0.005.

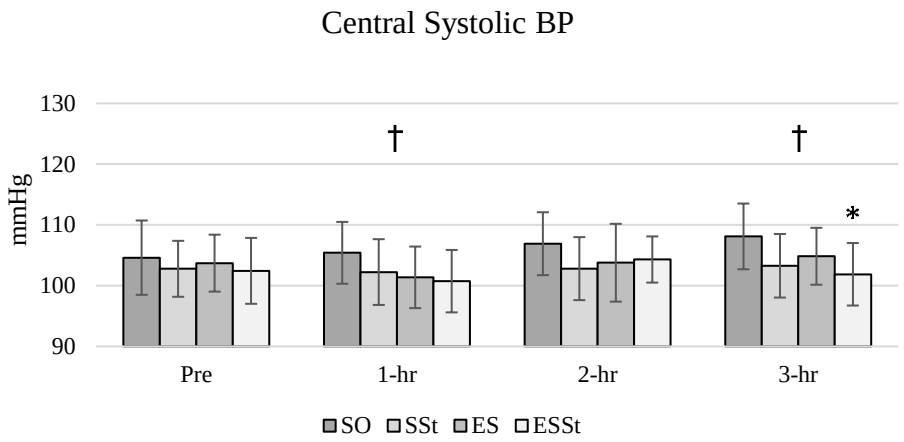


Figure 4.10 *Central Systolic BP – Condition Main Effects*

†Denotes significant individual main effects of condition at specific time point (p<0.05)

*Denotes significant difference from condition SO (p<0.05)

Individual main effects of condition showed a statistically significant difference in central systolic BP at 1-hour-intervention (F(3, 42)=4.480, p=0.008) and 2-hours-intervention (F(3, 42)=5.980, p=0.002). Therefore, Sidak-adjusted post-hoc planned pairwise comparisons were run. Data are mean ± standard error, unless otherwise stated. At 3-hours-intervention, central systolic BP was significantly higher during the Sitting Only condition (108.10 ± 2.399) compared to the Exercise Plus Sitting Plus Standing condition (101.87 ± 2.339), a significant difference of 6.233 (95% CI, 1.298 to 11.169) mmHg, p=0.010.

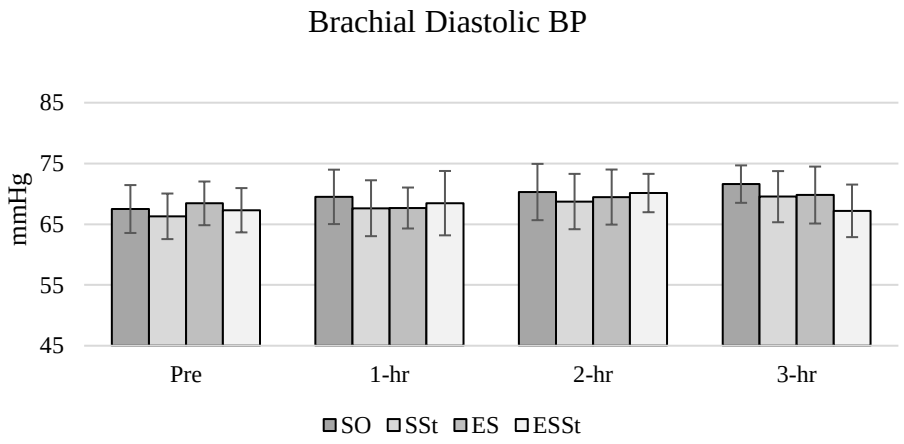


Figure 4.11 *Brachial Diastolic BP – Condition Main Effects*

Individual main effects of condition showed no statistically significant differences in brachial diastolic BP for any time points.

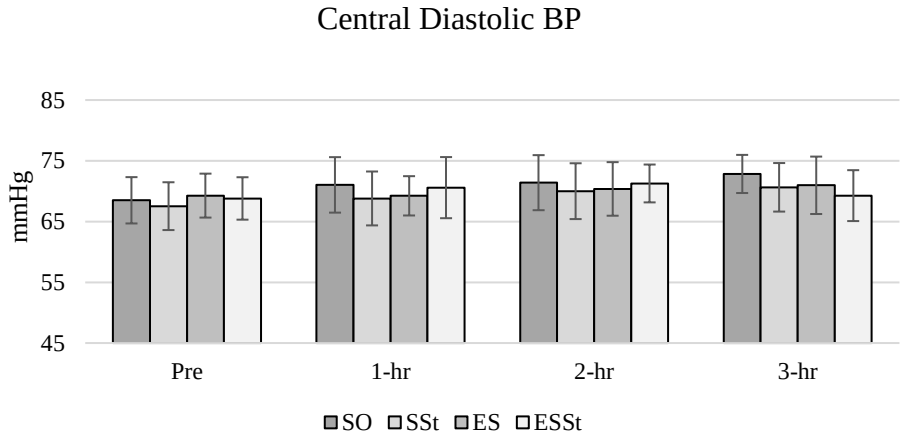


Figure 4.12 *Central Diastolic BP – Condition Main Effects*

Individual main effects of condition showed no statistically significant differences in central diastolic BP for any time points.

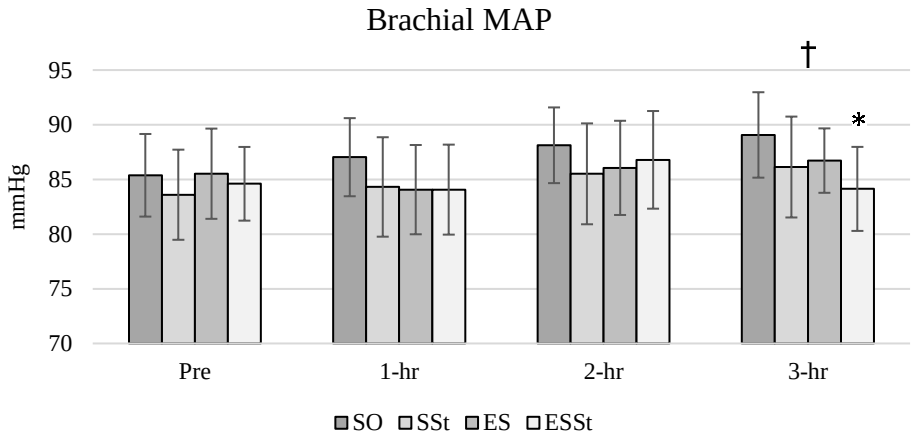


Figure 4.13 *Brachial MAP – Condition Main Effects*

†Denotes significant main effects of condition at specific time point ($p<0.05$)

*Denotes significant difference from condition SO ($p<0.05$)

Individual main effects of condition showed a statistically significant difference in brachial MAP at 3-hours-intervention ($F(3, 42)=4.326$, $p=0.010$). Therefore, Sidak adjusted post-hoc planned pairwise comparisons were run. Data are mean ± standard error, unless otherwise stated. At 3-hrs-intervention, brachial MAP was significantly higher during the Sitting Only condition (89.07 ± 1.571) compared to the Exercise Plus Sitting Plus Standing condition (84.13 ± 1.79), a significant difference of 4.933 (95% CI, 0.119 to 9.748) mmHg, $p=0.043$.

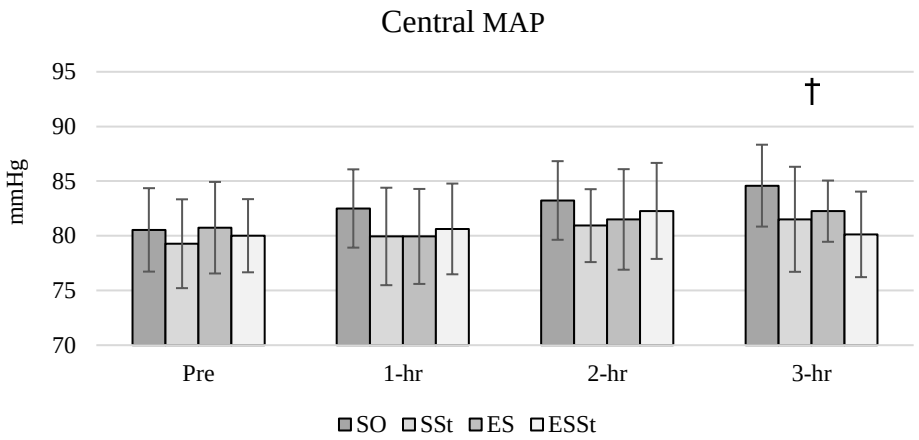


Figure 4.14 *Central MAP – Condition Main Effects*

†Denotes significant main effects of condition at specific time point ($p<0.05$)

Individual main effects of condition showed a statistically significant difference in central MAP at 3-hours-intervention ($F(3, 42)=4.326$, $p=0.010$). Therefore, Sidak adjusted post-hoc planned pairwise comparisons were run, and found no significant differences between any conditions.

4.2.3 Specific Aim 1 Follow-Up: To determine how sitting-induced vascular responses change with time within each condition.

The purpose of this analysis was to determine how the vascular parameters of brachial systolic BP, brachial diastolic BP, central systolic BP, and central diastolic BP, and cfPWV differ between the time points within each condition.

4.2.3.1 Carotid-Femoral PWV – Time Individual Main Effects

Individual main effects of time on cfPWV within each condition were analyzed using a paired-samples t-test. The differences in cfPWV between pre-intervention and

post-intervention were normally distributed for all conditions (p>0.05) as assessed using Shapiro-Wilk's test for normality. Individual main effects of time on cfPWV are displayed graphically in Figure 4.15.

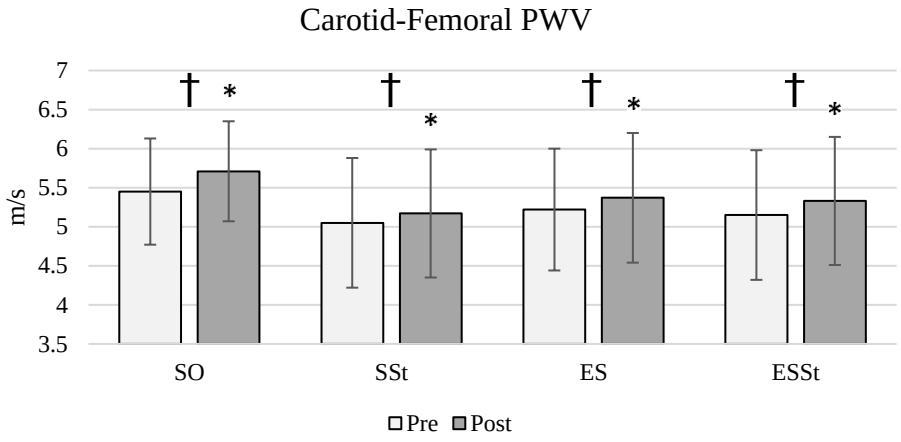


Figure 4.15 *Carotid-Femoral PWV – Time Main Effects*

†Denotes significant individual main effects of time within specific condition (p<0.05)

*Denotes significant difference from pre-intervention (p<0.05)

Individual main effects analysis of time found statistically significant increases in cfPWV from pre-intervention to post-intervention for all conditions. Therefore, Sidak-adjusted post-hoc pairwise comparisons were run. All data is presented as mean ± standard deviation, unless otherwise stated. For the Sitting Only condition, there was a significant increase from 5.45 m/s ± 0.68 at pre-intervention to 5.71 m/s ± 0.64 at post-intervention, a statistically significant increase of 0.26 (95% CI, 0.11 to 0.42) m/s, t(13)=3.69, p=0.003, d=0.99. For the Intermittent Standing condition, there was a significant increase from 5.05 m/s ± 0.83 at pre-intervention to 5.17 m/s ± 0.82 at post-intervention, a statistically significant increase of 0.11 (95% CI, 0.004 to 0.22) m/s,

t(14)=2.22, *p*=0.043, d=0.57. For the Exercise Plus Sitting condition, there was a significant increase from 5.22 m/s ± 0.78 at pre-intervention to 5.37 m/s ± 0.83 at post-intervention, a statistically significant increase of 0.16 (95% CI, 0.07 to 0.24) m/s, t(14)=3.99, *p*=0.001, d=1.03. For the Exercise Plus Sitting Plus Standing condition, there was a statistically significant increase from 5.15 m/s ± 0.83 at pre-intervention to 5.33 m/s ± 0.82 at post-intervention, a statistically significant increase of 0.18 (95% CI, 0.06 to 0.31) m/s, t(14)=3.071, *p*=0.008, *d*=0.793.

4.2.3.2 Blood Pressure – Time Individual Main Effects

Individual main effects of time on each BP variable within each condition were analyzed using a one-way repeated measures ANOVA. The assumption of sphericity was met for the main effects of time on all BP variables for each condition, except for central systolic BP for the Exercise Plus Sitting condition ($\chi^2(2)=12.735$, $p=0.026$). Results for individual main effects of time on BP parameters are displayed graphically in Figures 4.16, 4.17, 4.18, 4.19, 4.20, 4.21.

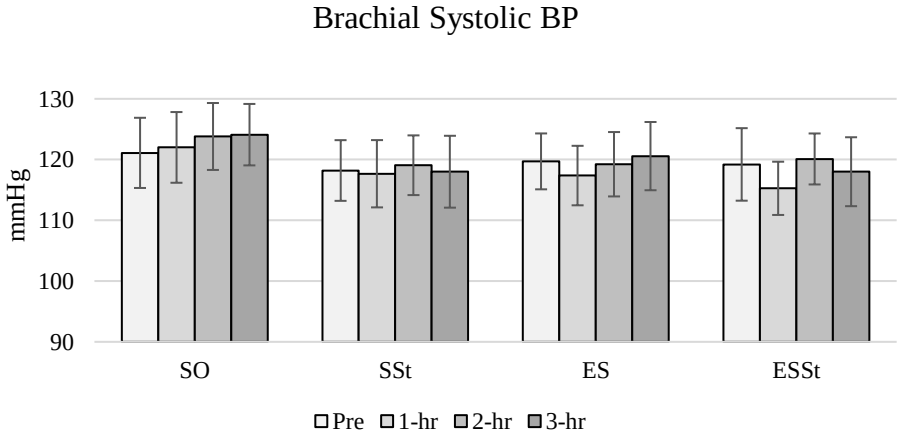


Figure 4.16 *Brachial Systolic BP – Time Main Effects*

Individual main effects of time showed no statistically significant differences in brachial systolic BP between any time points within any conditions.

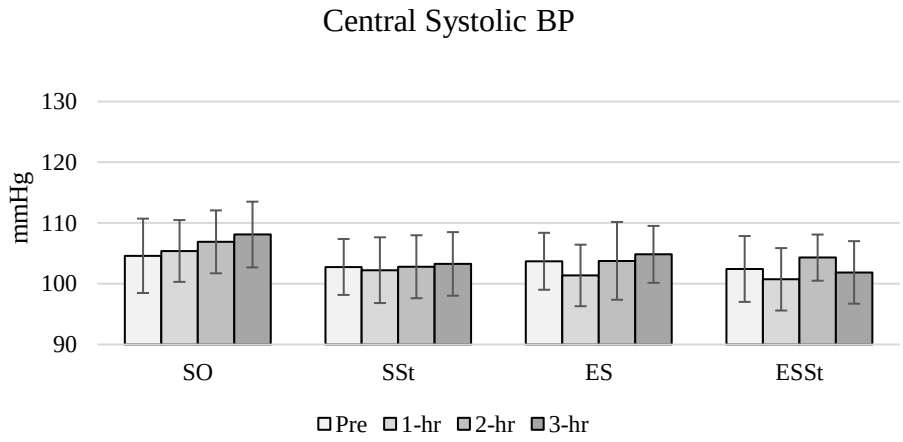


Figure 4.17 *Central Systolic BP – Time Main Effects*

Individual main effects of time showed no statistically significant differences in central systolic BP between any time points within any conditions.

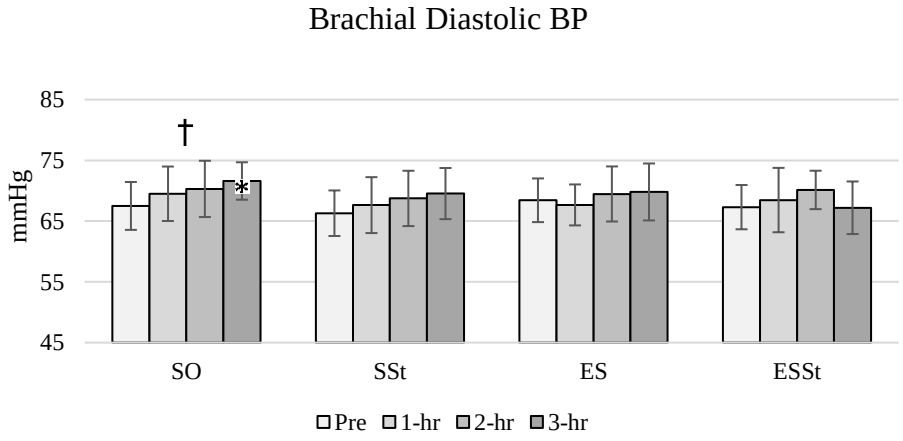


Figure 4.18 *Brachial Diastolic BP – Time Main Effects*

†Denotes significant individual main effects of time within specific condition ($p<0.05$)

*Denotes significant difference from pre-intervention ($p<0.05$)

Individual main effects of time showed a statistically significant difference in brachial diastolic BP within the Sitting Only condition ($F(3, 42)=3.087$, $p=0.037$). Therefore, Sidak adjusted post-hoc pairwise comparisons were run. Data are mean ± standard error, unless otherwise stated. There was a significant increase in brachial diastolic BP from 67.50 ± 1.838 mmHg at pre-intervention to 71.567 ± 1.437 mmHg at post-intervention, a statistically significant increase of 4.067 (95% CI, 0.083 to 8.051) mmHg, $p=0.044$.

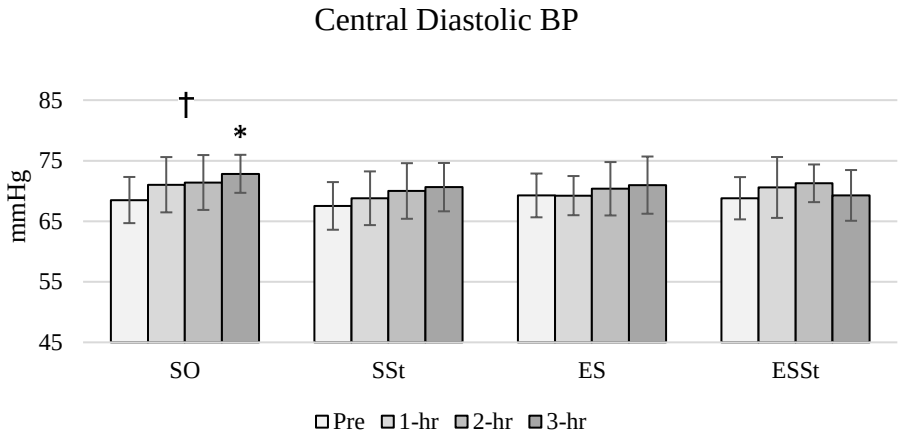


Figure 4.19 *Central Diastolic BP – Time Main Effects*

†Denotes significant individual main effects of time within specific condition ($p<0.05$)

*Denotes significant difference from pre-intervention ($p<0.05$)

Individual main effects of time showed a statistically significant difference in central diastolic BP within the Sitting Only condition ($F(3, 42)=3.848$, $p=0.016$). Therefore, Sidak adjusted post-hoc pairwise comparisons were run. Data are mean ± standard error, unless otherwise stated. There was a significant increase in central diastolic BP from 68.50 ± 1.778 mmHg at pre-intervention to 72.83 ± 1.464 mmHg at

post-intervention, a statistically significant increase of 4.333 (95% CI, 0.722 to 7.944) mmHg, p=0.015.

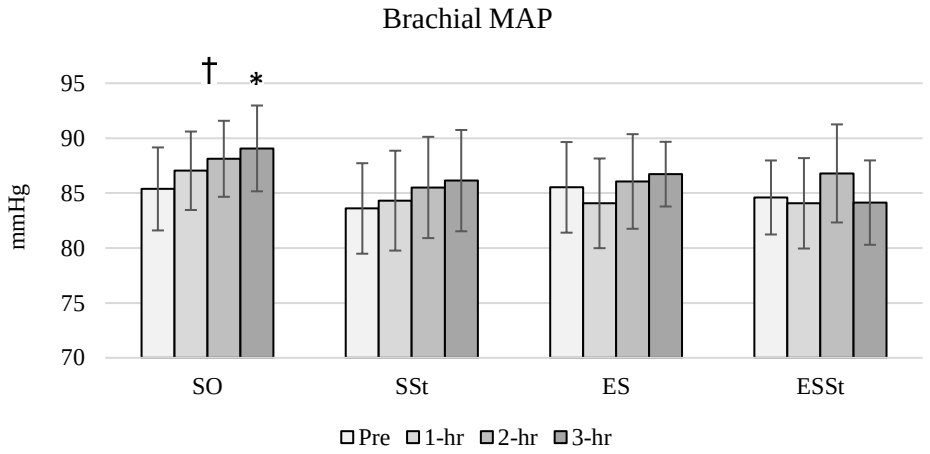


Figure 4.20 *Brachial MAP – Time Main Effects*

†Denotes significant individual main effects of time within specific condition (p<0.05)

* Denotes significant difference from pre-intervention (p<0.05)

Individual main effects of time showed a statistically significant difference in brachial MAP within the Sitting Only condition (F(3, 42)=3.644, p=0.020). Therefore, Sidak adjusted post-hoc pairwise comparisons were run. Data are mean ± standard error, unless otherwise stated. There was a significant increase in brachial MAP from 85.38 ± 1.76 mmHg at pre-intervention to 89.07 ± 1.57 mmHg at post-intervention, a statistically significant increase of 3.689 (95% CI, 0.123 to 7.255) mmHg, p=0.041.

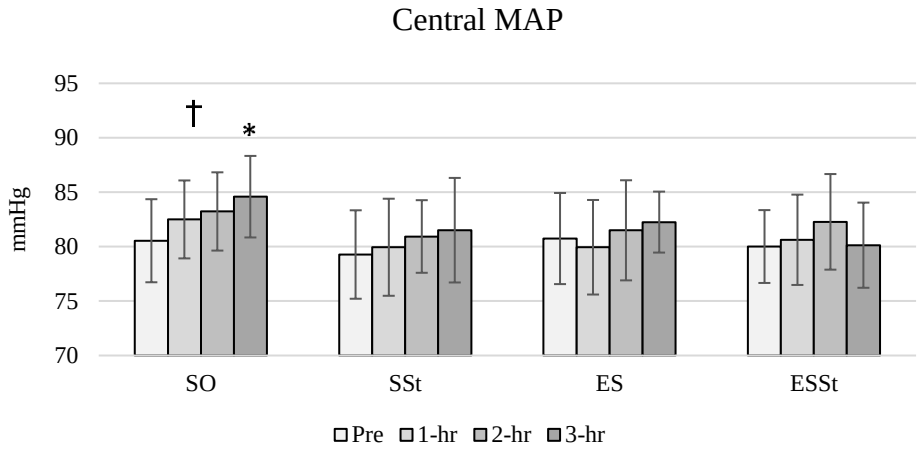


Figure 4.21 *Central MAP – Time Main Effects*

† Denotes significant individual main effects of time within specific condition ($p<0.05$)

* Denotes significant difference from pre-intervention ($p<0.05$)

Individual main effects of time showed a statistically significant difference in central MAP within the Sitting Only condition ($F(3, 42)=4.161$, $p=0.011$). Therefore, Sidak adjusted post-hoc pairwise comparisons were run. Data are mean ± standard error, unless otherwise stated. There was a significant increase in central MAP from 80.54 ± 1.78 mmHg at pre-intervention to 84.59 ± 1.56 mmHg at post-intervention, a statistically significant increase of 4.044 (95% CI, 1.091 to 6.998) mmHg, $p=0.005$.

CHAPTER V – DISCUSSION

5.1 Summary of Findings

The purpose of this study was to explore vascular responses to prolonged sitting, with or without the addition of strategies including intermittent standing and/or prior moderate intensity exercise. Previous literature has investigated the effects of prolonged sitting on PWV, BP and other vascular parameters, but few studies have investigated the effects of intermittent standing during prolonged sitting, or performance of exercise prior to prolonged siting on any vascular parameters, let alone cfPWV. Investigation into these interventions will therefore contribute to the current body of literature regarding the effectiveness of these strategies, and potentially provide meaningful health care strategies for the general population who are exposed to prolonged sitting, especially those in the workplace who are able to implement standing work desks. In addition, investigating both BP and cfPWV could potentially improve understanding of the mechanisms linking sedentary exposures to adverse vascular changes and CVD development, which is still not fully understood in current literature.

This study utilized a randomized-controlled repeated measures design, with participants completing four separate conditions on separate visits: Sitting Only (control), Sitting Plus Standing, Exercise Plus Sitting, and Exercise Plus Sitting Plus Standing. Brachial and central BP were measured in a sitting position at pre-, 1-hour-, 2-hours- and 3-hours-intervention, while cfPWV was measured in a supine position at pre- and post-intervention. It was hypothesized that brachial and central BP and cfPWV would be significantly increased from pre- to post-intervention for the Sitting Only (control)

condition and the Sitting Plus Standing condition, and these increases from pre- to post-intervention would be significantly greater than for the Exercise Plus Sitting and Exercise Plus Sitting Plus Standing conditions.

The primary aim of this study was to determine how vascular responses are affected over time by the sitting strategies utilized, which was met by analyzing interactions between the factors of condition and time for all variables. There were no statistically significant interactions across conditions for the variables of this studyThe second aim of this study was to analyze and compare the effectiveness of the strategies utilized for mitigating negative sitting-induced vascular responses, which was met by analyzing individual main effects of condition on each of the variables. The results showed a significant individual main effects by condition on cfPWV at pre-intervention and post intervention, brachial systolic BP at 1-hour-intervention, 2-hours-intervention, and 3-hours-intervention, and central systolic BP at 1-hour-intervention and 3-hours-intervention. However, pairwise comparisons found cfPWV was only statistically significantly higher for the Sitting Only condition compared to the Sitting Plus Standing condition at post-intervention, and brachial systolic BP at 1-hour intervention and central systolic BP at 3-hours intervention was significantly higher for the Sitting Only condition compared to the Exercise Plus Sitting Plus Standing condition. A follow up analysis to the first aim investigated individual main effects of time within each condition. There were significant main effects of time for cfPWV within all conditions, and for brachial diastolic and central diastolic BP within the Sitting Only condition. Pairwise comparisons revealed significant increases in cfPWV from pre- to post-intervention

within all conditions, and significant increases in brachial diastolic and central diastolic BP from pre- to post-intervention within the Sitting Only condition.

5.2 Acute Effects of Prolonged Sitting Time With and Without Intermittent Standing And/Or Prior Exercise on Carotid-Femoral PWV

One of the primary findings of this study was that the effects of sitting duration on central-femoral PWV was not dependent on the type of intervention condition utilized, which included sitting with and without intermittent standing, and/or prior exercise. The study hypotheses would presuppose significant interactions between condition and time, with the first hypothesis proposing significant increases in cf-PWV from pre- to post-intervention for the Sitting Only and Sitting Plus Standing conditions, and the second hypothesis proposing cfPWV at post-intervention would be significantly greater for the Sitting Only and Sitting Plus Standing conditions compared to the Exercise Plus Sitting and Exercise Plus Sitting Plus Standing conditions. These hypotheses were therefore rejected, however,the evidence from which they were formulated is scarce. Previous literature has found prolonged sitting leads to acute increases in cfPWV – an indicator of aortic vascular stiffening (Credeur et al., 2018), but the evidence for the effectiveness of intermittent standing and prior exercise interventions on cfPWV are limited. A study by Gibbs et al. (2017) found cf-PWV responses do not differ between intermittent standing (for 30-minutes every 30-minutes) and control conditions in overweight/obese individuals, and Kruse et al. (2018) found intermittent standing for 10-minutes every 30-minutes over a 4-hour sitting duration to provide no benefit for increasing flow mediated dilation in the lower extremities compared to a control condition. Regarding prior

exercise, a study by Perdomo et al. (2016) investigated 24-hour post-exercise changes in cfPWV, finding 30-minutes of moderate to vigorous aerobic exercise to significantly reduce cfPWV. Huang et al. (2015) also found chronic reductions in peripheral and central PWV from aerobic endurance training. To the researchers knowledge,no studies have looked at the acute influence of prior exercise during prolonged sitting on cfPWV. The effectiveness of exercise for lowering cfPWV, acutely may therefore be questionable, and discussion of individual main effects of condition and time will provide more detail to these findings and perhaps identify potential causes.

Further analysis into the individual main effects of condition found cfPWV was significantly different between conditions at pre-intervention and post-intervention, but further comparisons foundthe Sitting Only condition to be significantly higher than the Sitting Plus Standing condition at post-intervention. This may indicate potential benefit for the intermittent standing strategy in reducing elevations in cfPWV due to sitting, however, this conclusion should not be implied with high confidence considering this analysis didn't compare overall changes in cfPWV and the measures at pre-intervention were significantly different between conditions. Although it was proposed that intermittent standing would have similar overall cfPWV to sitting only, which would potentially conflict this finding, this hypothesis cannot be refuted based on these findings due to the nature of this analysis. Evidence by Gibbs et al. (2018) found intermittent standing for 30-minutes every 30-minutes over a 4-hour sitting duration was not effective for mitigating elevations in cfPWV and would provide support that intermittent standing should not be given credit for reducing cfPWV.

The significant increases in cfPWV from pre- to post-intervention for all conditions however suggest the strategies of intermittent standing, prior exercise or a combination of both strategies may not be effective for offsetting the significant elevations in cfPWV after 3-hours of prolonged sitting. This finding rejects the study hypotheses in respect to the exercise conditions, that were proposed to mitigate the increases in cfPWV from sitting, based on the previous literature that found the performance of 45-minutes of aerobic exercise at 65% of VO_2 maximum prior to sitting to prevent sitting-induced reductions in flow-mediated dilation in the lower extremities (Ballard et al., 2017;Morishima et al., 2017), and the expected acute reductions in BP from exercise (Carpio-Rivera et al., 2016; Green & Smith, 2018) that would likely reduce cf-PWV. The study hypotheses, however, presupposed an association between cfPWV and FMD, which could be questioned within the scientific literature (Koivistoinen et al., 2012; Kobayashi et al., 2004). Systolic BP has, however, been found to be positively correlated with pre- and post-exercise cfPWV in previous evidence (Perdomo et al., 2016). Furthermore, the measures of FMD by Ballard et al. (2017) and Morishima et al. (2017) were also in the lower extremities, which could be influenced through different sitting-induced mechanisms from the upper and central-extremity vasculature, such as blood pooling due to a lack of venous return (Restaino et al., 2015). In contrast, the present study may suggest that longer durations or higher intensities of aerobic exercise are needed prior to sitting to meaningfully offset elevations in PWV, as found in previous literature on FMD (Ballard et al., 2017; Morishima et al., 2018). This is partially supported by a meta-analytic review by Huang et al. (2015) that found longer durations of aerobic exercise training to be more effective for chronically reducing PWV,

especially in the central vasculature. Although the elevated cfPWV could be from the intermittent standing, which ceased about 30-minutes before post-intervention measurements, this would seem unlikely considering cfPWV was still significantly elevated from the Exercise Plus Sitting condition which did not utilize intermittent standing. Identification of cfPWV throughout the 3-hour sitting duration would have been desirable to detect specific acute effects of exercise or standing, however, this was not possible considering this measurement required a supine position and would have required postural deviations from the sitting position.

5.3 Acute Effect of Prolonged Sitting Time with and without Intermittent Standing and/or Prior Exercise on Blood Pressure

The second major finding of this study was the effects of sitting duration on all BP variables were not dependent on the type of intervention condition utilized, which included 3-hours of sitting with and without intermittent standing, and/or prior exercise. The first study hypothesis proposed significant increases in BP from pre- to post-intervention for the Sitting Only and Sitting Plus Standing conditions, while the second study hypothesis proposed BP at post-intervention for these conditions would be significantly greater compared to the Exercise Plus Sitting and Exercise Plus Sitting Plus Standing conditions. Both these hypotheses were rejected, since these findings would require the vascular responses to be dependent on the type of strategy that is utilized and the duration of its implementation. Given the existing evidence for the effectiveness of acute exercise performance on reducing BP for up to 24 hours (Carpio-Rivera et al., 2016; Green & Smith, 2018) and elevating flow-mediated dilation for up to 3-hours after

it's cessation (Ballard et al., 2017; Morishima et al., 2018), and the evidence for prolonged sitting acutely increasing peripheral BP over time (Paterson et al., 2021), it was reasonable to expect both brachial and central BP responses over sitting duration to be positively influenced by the conditions utilizing moderate intensity exercise, especially in comparison to the control condition. However, the present findings suggest no influence on BP responses to sitting at 1-hour, 2-hours, and 3-hours depending on whether prolonged sitting is accompanied with intermittent standing and/or prior exercise strategies. Although the cause of a lack of interaction cannot be directly conferred, the data would seem to present a high standard error for the degree of variation in BP due to time or condition, which could be a contributing factor. This error may be due to several factors, but variation in health or demographic status would seem possible, which could produce varied physiological responses to sitting and/or the strategies utilized. Although few studies have investigated differences in sitting-induced BP responses due to cardiovascular risk factors, evidence may suggest differences in exercise-induced responses. A systematic review by Cardoso et al. (2010) concluded that ambulatory BP after aerobic exercise was only sometimes hypotensive in normotensive subjects but was significantly decreased most of the time in hypertensive individuals. A systematic review and meta-analysis by Hamer et al. (2006) however found no differences due to fitness level. The study participants had an average BMI of 27.8 ± 7.3 kg/m^2 (nine normal weight and six overweight/obese) and PA level score of 55 ± 21.5 (classed as active), which would seem to pose a large variation in these parameters but may have no influence on sitting- or exercise-induced vascular responses based on current evidence. The variations could therefore perhaps be attributed to race, with eight of the participants

being African American and seven being White or other. Only two of the participants in the present study would meet the criteria for hypertension by the AHA (Arnett et al., 2019) based on resting brachial systolic BP values recorded greater or above 130 on two separate visits, and would therefore be unlikely to produce variable data overall.

There were significant individual main effects of condition on brachial and central systolic BP at 1-hour- and 3-hours-intervention and brachial systolic BP at 2-hours-intervention, but with further comparisons only showing the Sitting Only condition to be significantly higher than the Exercise Plus Sitting Plus Standing condition at 1-hour-intervention for brachial systolic BP and 3-hours-intervention for central systolic BP. These findings could imply that the prior exercise and intermittent standing in combination are effective for reducing brachial systolic BP after one hour of sitting, or central systolic BP after 3-hours of sitting, compared to sitting with no strategies. This conclusion should, however, be cautiously provided given this analysis didn't take baseline magnitudes or total BP changes into account, and only compared differences between conditions at each time point. Baseline measures were, however, not significantly different between conditions, which may strengthen the prior conclusion. These findings would seem to partially support the study's second hypothesis that the prior exercise strategies would mitigate elevations in systolic BP after 3-hours of sitting, although the present findings lack significance for the prior exercise strategy alone. To the researchers' knowledge, this novel strategy of prior exercise in combination with intermittent standing has never been tested in previous literature but was hypothesized to reduce BP based on the evidence for the well-established hypotensive effects of exercise, and the evidence for prior exercise by Ballard et al. (2017) and Morishima et al. (2018),

who found 45 minutes of aerobic exercise at around 65% of maximum oxygen consumption to mitigate reductions in FMD in the lower extremities after 3-hours of sitting. The finding could present a potential benefit of intermittent standing when added to prior exercise for reducing diastolic BP, with a lack of significance for prior exercise alone. This evidence would not be supported by Gibbs et al. (2018) which found 30-minute standing durations every 30-minutes was not effective for reducing systolic BP in the brachial arteries over a 10-hour duration of sitting.

Further analyses indicated individual main effects of time on brachial and central diastolic BP within the Sitting Only condition, which elicited significant increases in these parameters from pre- to post-intervention. This may demonstrate that the duration of sitting perhaps might influence diastolic BP in the brachial and aortic arteries when sitting is not accompanied by intervention strategies, and systolic BP does not increase significantly over 3-hours of sitting. It may also demonstrate that accompanying sitting with intermittent standing and/or prior exercise prevents significant increases in diastolic BP in the brachial and aortic arteries. This finding partly refutes the study's first hypothesis that 3-hours of sitting with no strategies would significantly increase all BP variables and the strategies containing exercise would prevent these sitting-induced increases in the BP variables. Although systolic BP appeared to gradually increase during the Sitting Only condition, this did not reach the statistical significance that was expected from previous literature (Paterson et al., 2021). This may have been due to high standard errors, which could have stemmed from the outliers within this separate one-way repeated measures analyses. This was demonstrated whereby participant (n=1) was an outlier for all brachial and central BP responses which was not identified to be due to

factors outside of individual physiology. The potential vascular health benefit of intermittent standing is again demonstrated, which refutes the first study hypothesis that proposed no effects, while supporting previous findings by Gibbs et al. (2017) that found significant reductions in diastolic BP from intermittent standing for 30-minute durations every 30-minutes over a 10-hour period of prolonged siting. These benefits of intermittent standing would perhaps stem from the increased postural changes and skeletal muscle activation from standing that can increase blood flow (Olufsen et al., 2005), and possibly mitigate the apparent reductions in peripheral blood flow that can occur during prolonged sitting (Restaino et al., 2015). The potential vascular health benefits of prior exercise in the present study are consistent with the study hypothesis and support previous data for exercise reducing BP compared to prolonged sitting (Paterson et al., 2021). The expected hypotensive effects of exercise from previous literature (Carpio-Rivera et al., 2016; Green & Smith, 2018) were however not statistically significant within the present study which is surprising based on the previous evidence. The reasons for a lack of significance can perhaps be attributed to the variations in participants' physiological responses to sitting or exercise as previously discussed. Standard errors were around 2-3 mmHg and with changes in BP ranging from 2-4 mmHg between time points.

5.4 Association Between Carotid-Femoral PWV And Blood Pressure Parameters

The present study found significant increases in cfPWV across all conditions, while the only BP parameters to exhibit significant increases over time for any conditions was brachial and central diastolic BP for the Sitting Only (control) condition. Although

the present study did not analyze associations between the changes in BP and cfPWV, some possible conclusions can be made from these findings. As previously discussed, the strategies utilizing prior exercise were expected to be preventative of significant increases in cfPWV over time from sitting, with the assumption that elevations in FMD or reductions in BP from prior exercise compared to uninterrupted sitting would effectively mitigate elevations in cfPWV. The metabolic demands of exercise would vasodilate the arteries, increasing the diameter of the artery lumen and provide more space for blood to flow through the periphery and to working skeletal muscle. The increases in blood flow from exercise would be expected to gravitate towards resting levels within 3-hours of sitting, but with a wider artery diameter compared to a 3-hour sitting bout with no prior exercise (Ballard et al., 2017), due to maintained artery vasodilation from exercise (Halliwill., 2001). This would likely exhibit decreases in the velocity of blood flow through the arteries at 3-hours of sitting if the lumen is wider and blood flow had returned to baseline. However, the findings of this study suggest otherwise, with significant elevations in cfPWV for all conditions not being accompanied by increases in any BP parameters other than diastolic BP in the brachial and aortic arteries for the control condition. This may suggest that BP in the brachial or aortic arteries has little relation or influence on cfPWV, and other mechanisms or vascular changes are responsible. An alternative explanation is the exercise or standing within the strategy conditions did not elicit strong enough artery vasodilation to make a meaningful difference in cfPWV after 3-hours of sitting, which may be possible considering the previous evidence by Ballard et al. (2017) and Morishima et al. (2018) utilized 45-minutes of vigorous aerobic exercise as opposed to the 30-minutes of moderate aerobic exercise or intermittent standing utilized

in this study.It's been previously shown thiscan affect the degree of post-exercise hypotension (Halliwill., 2001). In contrast to this explanation, the Exercise Plus Sitting Plus Standing condition elicited significantly lower brachial systolic BP compared to the control condition at 3-hours of sitting, which may indicate meaningful dilation of the brachial artery. More vascular parameters would need to be collected to determine the possible mechanisms behind the acute elevations in cfPWV from sitting, and whether it should be considered an adverse vascular consequence that contributes to CVD development.

5.5 Implications

Although the present study did not find vascular changes due to sitting duration to be significantly dependent on the strategies of intermittent standing and/or prior exercise, potential individual benefits of intermittent standing and/or prior exercise were found for offsetting elevations in diastolic BP in the brachial and aortic arteries, and a combination of intermittent standing and prior exercise for reducing systolic BP in these arteries after 1- or 3-hours of sitting. Systolic and diastolic BP have been shown to have clinical significance in health care, with values over 130 mmHg and 80 mmHg respectively being part of the diagnosis of hypertension (Arnett et al., 2019) – a risk factor for CVD (Flint et al., 2019; Franklin & Wong., 2013). If performing intermittent standing during sitting or performing exercise prior to sitting have the potential to acutely mitigate increases in diastolic BP, or if a combination of these strategies potentially reduces systolic BP during sitting, then this perhaps could provide meaningful health benefits over time. This would present value for populations that are often exposed to prolonged sitting and are able to

utilize sit-to-stand work desks or perform exercise prior to sitting, such as office workers which comprise a large majority of the population.

Furthermore, the findings for the lack of effectiveness of the intermittent standing and/or prior exercise strategies for offsetting elevations in PWV from the carotid to the femoral artery can still provide meaningful information for health care. Although these findings are not with high certainty due to the lack of condition-time interaction, they potentially highlight the need for greater durations of standing or prior exercise, or higher intensities of prior exercise, to offset the adverse vascular health changes from sitting. This finding is important, since it is likely that acute sitting-induced elevations in systolic BP are not desirable for health, with a pathway for hypertension likely resulting from indirect mechanisms associated with accumulated sedentary behavior over the life span (Guo et al., 2020; Lee & Wong, 2015). However, it is still unknown whether acute sitting-induced elevations in cfPWV are adverse for health and linked with cardiovascular disease (Park et al., 2020; Paterson et al., 2020).

5.6 Strengths and Limitations

The repeated measures design of the present study enabled the measurement of participants over all conditions and time points, which have relatively larger power in statistical analysis, due to the control for separate individual differences. It was also beneficial because it allowed the analysis of the effects of multiple conditions on multiple parameters, which was able to produce a lot of information from one experiment. Regarding the study protocol, measuring multiple time points allowed a clearer picture of the influence of sitting and the strategies utilized on the vascular health parameters over

time, while the 3-hour total condition duration provided an appropriate and realistic time frame to measure BP and arterial stiffening responses. Readings of BP were also taken via the SphgoCor Xcel device which would likely have more reliability and accuracy than taking measurements manually due to human error. The recruitment of fifteen participants was a decent number for this type of analysis to draw meaningful conclusions about the data, although the strength of the analysis could have been improved from having more participants. The study also successfully controlled external variables that could affect the measurements, such as asking participants to abstain from caffeine and nicotine consumption 12-hours prior to the start of visits as well as food consumption for 2-hours, making results more trustworthy. The results would seem to be highly generalizable to a large population who have the option to utilize sit-to-stand work desks in the workplace. The present study also investigated various conditions and variables that are novel in scientific literature, such as the effects of a combination of prior exercise and intermittent standing on cfPWV and contributing scientific evidence towards strategies that have been less commonly utilized, such as the effects of prior exercise and intermittent standing during sitting on the peripheral and central vasculature.

One of the first study limitations was identified after extensive data collection for the conditions utilizing standing, whereby elevations in BP seemed to be apparent at the 2-hour-intervention time point of measurement when visually assessing graph trend lines. Although these values were not classed as outliers (values greater than ± 3 standard deviations), two of these measurements violated the assumption of normality but were not transformed. The proposed cause of these elevated BP responses is extremely likely to be due to timing of the scheduled BP measurement (every-1-hour) falling at the same

instance as the end of the scheduled 10-minute standing bout. Evidence would suggest that BP can rise after standing (Olufsen et al., 2005), which would explain why BP recordings at 2-hours-intervention were higher than what they should have been for the intermittent standing conditions. These alterations in BP due to posture should have been considered and controlled by utilizing postural stabilization periods, to get a more accurate measurement for the underlying resting BP responses due to the strategies, as utilized in an experiment by Gibbs et al (2017). More vigorous pilot testing could have spotted this limitation earlier and should be a consideration in future experiments. This limitation may not have influenced the analysis for the individual effects of time considering the BP measurement was also administered at post-intervention but may have done when analyzing time-condition interactions.

Other limitations relate to the control of variables outside of the study setting. Controlling PA prior to the study posed a challenge and could have influenced the findings. Although participants were encouraged to minimize vigorous intensity exercise on the day prior to the visits and successfully did so according to their reports, moderate intensity exercise would have been hard to limit and could have potentially elicited unique vascular alterations in sedentary individuals if long durations had been performed, and blunted BP responses during the study. This was especially true where participants were often required to walk several hundred meters to the study location on the day of their visit, which could have elicited initial elevations in sympathetic activity during the study visits. Although the investigator gave extra rest time when necessary for measurements at baseline, BP measurements at baseline could have been slightly elevated above rest due to this activity.

It is also important to note that the SphygmoCor AtCor Medical BP cuff (23-33 cm) had to be replaced with a SunTech BP cuff (26-34 cm) during week 5 of the study due to a leak, which resulted in data being obtained from both cuffs in 6 participants. However, when resting BP values were compared between successive visits using the same cuff vs different cuffs, no significant differences were observed for systolic and diastolic BP and MAP (all $p \geq 0.61$). Therefore, this appeared to have very little, if any, influence on the final interpretation of the results.

5.7 Future Directions

With the present study finding little to no statistical significance for the effectiveness of prior exercise and intermittent standing as strategies to offset the adverse vascular changes from prolonged sitting, more research should be conducted to verify these findings, perhaps with less condition groups and testing one strategy at a time vs a control group to look at individual effects. It would seem relatively urgent to confirm the effectiveness of the intermittent standing strategy, with implementation being feasible for those who possess sit-stand work desks. Experimentation on longer duration standing bouts, higher frequencies of standing, or standing vs sitting strategies could be implemented to find meaningful doses. The acute effects of prior exercise on PWV during sitting need further investigation, considering chronic aerobic endurance training has been found to effectively reduce cf-PWV over time (Huang et al., 2015). The effects of higher intensity or longer duration exercise performance prior to sitting could also be explored. To investigate physiological mechanisms behind these strategies, future work should measure more vascular parameters and examine how they relate to arterial

stiffening. For example, a BP index could identify exact BP changes with time and provide more clear information for whether BP parameters are reduced over prolonged siting compared to control groups alone. Most studies have also measured vascular responses in the lower extremities while more work should be focused on the upper extremity and central vasculature, which can still provide clinical implications for health and future disease (Niiranen et al., 2019; Roman et al., 2009; Zhong et al., 2018).

Future analysis should also explore influences of cardiovascular risk factors, race, BMI or physical activity level on sitting-induced vascular health responses. Control for race or health status via stratification of BMI or resting BP is worth implementing in future works.

5.8 Summary

It is well established that prolonged sitting is a form of sedentary behavior that is adverse for acute vascular health outcomes in the lower extremity vasculature. Blood pooling and reductions in blood flow-induced shear stress from sedentary behavior can lead to epithelial dysfunction – a possible contributing mechanism for later CVD development (Thosar et al., 2012). The present study demonstrates increases in cfPWV and brachial and central diastolic BP over a 3-hour duration of uninterrupted sitting, which may also suggest negative vascular health outcomes from prolonged sitting in the upper extremities. More research is needed to determine whether sitting-induced increases in PWV is a prognosis for future cardiovascular disease, which could contribute to a better understanding for how prolonged sedentary exposures may lead to cardiovascular disease.

The health benefits of exercise or physical activity are well-established in the existing literature and interrupting prolonged sitting with moderate intensity aerobic exercise has been shown as an effective strategy for mitigating sitting-induced reductions in FMD in the lower extremities. However, this strategy may pose a challenge for implementing in the workplace, which presents the need for more research on the performance of prior exercise or standing interruptions, which this thesis investigated. The present study did not find prior exercise, intermittent standing, or a combination of these strategies to effectively mitigate elevations in cfPWV from prolonged sitting, although they might be effective for preventing increases in diastolic BP in the upper extremities, or a combination of prior exercise and intermittent standing might be effective for reducing upper extremity systolic BP after 1-hour or 3-hours of sitting compared to sitting alone. More research is needed on these types of strategies, especially considering they may be less challenging to implement than exercise interruptions in addition to the recent popularity of sit-stand work desks.

Appendix A – IRB Approval Form

Office of Research Integrity
THE UNIVERSITY OF SOUTHERN MISSISSIPPI
118 COLLEGE DRIVE #5125 • HATTIESBURG, MS | 601.266.6576 | USM.EDU/ORI

NOTICE OF INSTITUTIONAL REVIEW BOARD ACTION

The project below has been reviewed by The University of Southern Mississippi Institutional Review Board in accordance with Federal Drug Administration regulations (21 CFR 26, 111), Department of Health and Human Services regulations (45 CFR Part 46), and University Policy to ensure:

- The risks to subjects are minimized and reasonable in relation to the anticipated benefits.
- The selection of subjects is equitable.
- Informed consent is adequate and appropriately documented.
- Where appropriate, the research plan makes adequate provisions for monitoring the data collected to ensure the safety of the subjects.
- Where appropriate, there are adequate provisions to protect the privacy of subjects and to maintain the confidentiality of all data.
- Appropriate additional safeguards have been included to protect vulnerable subjects.
- Any unanticipated, serious, or continuing problems encountered involving risks to subjects must be reported immediately. Problems should be reported to ORI via the Incident template on Cayuse IRB.
- The period of approval is twelve months. An application for renewal must be submitted for projects exceeding twelve months.

PROTOCOL NUMBER: IRB-21-316
PROJECT TITLE: Intermittent Standing and Prior Exercise as Strategies to Interrupt Prolonged Sitting
SCHOOL/PROGRAM: Kinesiology
RESEARCHER(S): Alexander Wright, Stephanie McCoy

IRB COMMITTEE ACTION: Approved
CATEGORY: Expedited
4. Collection of data through noninvasive procedures (not involving general anesthesia or sedation) routinely employed in clinical practice, excluding procedures involving x-rays or microwaves. Where medical devices are employed, they must be cleared/approved for marketing. (Studies intended to evaluate the safety and effectiveness of the medical device are not generally eligible for expedited review, including studies of cleared medical devices for new indications.)

PERIOD OF APPROVAL: September 14, 2021

Donald Sacco Jr.

Donald Sacco, Ph.D.
Institutional Review Board Chairperson

Appendix B – Flyer

PARTICIPATE AND GET PAID!

For a study examining the effects of standing and walking on blood flow during prolonged sitting.

CAN YOU PARTICIPATE?
YOU MUST BE…

- ✓ Male between the ages of 18-45
- ✓ Free from cardiovascular, metabolic, neurological disease
- ✓ Not using tobacco products
- ✓ Able to attend 4 separate 4-hr visits over the semester ***(you will be allowed to do homework!)***

GENERAL PROCEDURES

- ❑ Informed Consent and Medical History
- ❑ Non-invasive measures of blood pressure and arterial stiffness
- ❑ Sitting for 3-hours with standing interruptions or walking prior to sitting
- ❑ Bring your homework!

SCAN THE QR CODE USING YOUR PHONE CAMERA OR FOLLOW THE LINK BELOW TO GET STARTED!

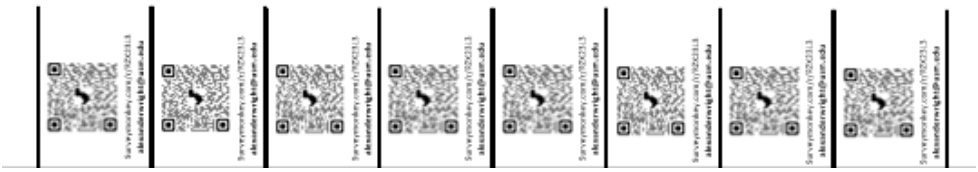
surveymonkey.com/r/RZX23L3

OR CONTACT ALEX WRIGHT FOR MORE INFORMATION

alexander.wright@usm.edu

The School of Kinesiology & Nutrition

THE UNIVERSITY OF SOUTHERN MISSISSIPPI

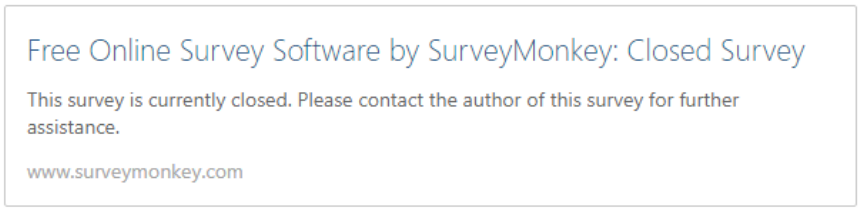


Appendix C – Recruitment Email

Dear Students,

A graduate student Alexander Wright is conducting a research study at the School of Kinesiology and Nutrition at USM to determine the effects of standing and walking on blood flow during sitting. Participants who complete the study successfully will be provided financial incentive. Would you be interested in participating in this study? Follow the link below to take a 1-minute survey to see if you are eligible!

https://www.surveymonkey.com/r/RZX23L3



To give you a bit more information, he will be measuring how your blood flow responds to 3-hours of prolonged sitting, with and without standing interruptions and/or prior exercise. The measurements are completely non-invasive. This study consists of 4 visits which include: 1) completion of an informed consent, a health history and physical activity questionnaire; and 2) four experimental sessions in which you will be asked to sit for a 3-hour duration with or without a standing or exercise strategy mentioned above. He will measure cardiovascular outcomes of blood pressure and arterial stiffness before, during and after sitting. Each visit may take up to 4 hours to complete, but you will be encouraged to complete your school/remote work during the study. You will also gain financial benefit and be paid $100 via a gift card for completion of all four of the required visits.

This study has been reviewed and approved by the University of Southern Mississippi Institutional Review Board (IRB-21-316).

If you have any questions, please let Alex know.

His email: alexander.wright@usm.edu
His phone: 603-369-8069

Appendix D – Recruitment Survey

Research Eligibility

Research Eligibility

Answer the following questions and we will determine whether you are eligible to participate in the study. We will contact you at the email address you provide.

1. What is your gender? (If you are female or other then unfortunately you cannot participate in this study, and please do not complete the rest of this questionnaire).

- ◯ Male
- ◯ Female
- ◯ Other

2. Are you between the ages of 18 and 45 years?

- ◯ Yes
- ◯ No

3. Do you currently, or have you previously had any cardiovascular, metabolic or neurological disease?

- ◯ Yes
- ◯ No

4. Do you currently smoke or use tobacco products, or have you stopped smocking within the last 6 months?

- ◯ Yes
- ◯ No

5. Would you currently consider yourself an elite athlete?

- ◯ Yes
- ◯ No
- ◯ Not Sure

6. Do you currently have a musculoskeletal injury or moderate back pain?

- ◯ Yes
- ◯ No
- ◯ Not Sure

7. Will you be able to attend 4 separate visits of 4 hours in duration (on campus over the course of the Fall semester) ?

- ◯ Definitely Can
- ◯ Probably Can
- ◯ Maybe
- ◯ Probably Cannot
- ◯ Definitely Cannot
- ◯ Not Sure

8. Are you currently an employee at the University of Southern Mississippi? (If you are then unfortunately we cannot pay you, although we'd still love you to participate!)

- ◯ Yes
- ◯ No

9. Please provide an email address at which you can be contacted. Your school email address is preferred.

Submit

Appendix E – Informed Consent

ORI | Office of Research Integrity

INSTITUTIONAL REVIEW BOARD

STANDARD (SIGNED) INFORMED CONSENT

STANDARD (SIGNED) INFORMED CONSENT PROCEDURES
• ***Use of this template is optional.*** However, by federal regulations (45 CFR 46.116), all consent documentation must address each of the required elements listed below (purpose, procedures, duration, benefits, risks, alternative procedures, confidentiality, whom to contact in case of injury, and a statement that participation is voluntary). • Signed copies of the consent form should be provided to all participants. Last Edited July 7th, 2021

Today's date:		
PROJECT INFORMATION		
Project Title: Intermittent standing and prior exercise as strateges to prevent arterial sitffening that can emanate from prolonged sitting.		
Principal Investigator: Alexander Wright	Phone: 603-369-8089	Email: alexander.wright@usm.edu
College: Education and Human Sciences	School and Program: University of Southern Mississippi. Kinesiology and Nutrition.	

RESEARCH DESCRIPTION

1. **Purpose**:

The purpose of this study is to explore the vascular health response to 3-hours of prolonged sitting, with and without standing interutpions and/or prior exercise. By examining and comparing these strategies, it can be determined whether standing interuptions and/or prior exercise can prevent arterial sitffening that can occur from prolonged durations of sitting. This research could help improve the health of individuals who spend a lot of time sitting in their workplace, which is a vast majority of the USA population.

2. **Description of Study**:

This study will require 4 separate visits of 3.5 to 4 hours in duration. I have the right to withdraw from the study and stop participating at any time. I may lose my financial benefits from the study but am still entitled to seek health care upon my withdrawal.

Please see a detailed description of the procedures for the study below.

Initial Visit:

I will report to the research laboratory (room 339 of Joseph Green Hall) at my scheduled time at the start of my first visit and review the inclusion and exclusion criteria of the study as well as the procedures and the potential risks and benefits of participating. If I agree to participate in the study, I will sign and provide my informed consent. I will then receive a medical history form which is to be completed immediately, and a physical activity questionairre which can be completed at any time during the intervention. At the start of future visits I will be able to undertake pre-intervention assessment procedures immediately.

It is estimated that I am one of twelve to fifteen participants in this study, and I will agree to abstain from caffeine, nicotine, or alchohol 12-hours prior to the start of any visit, will consume a meal at least 2 or more hours prior to the start of any visit, and abstain from vigorous intensity exercise 24-hours prior to the start of any visit.

Pre-Intervention Assessment:

An investigator will take pre-intervention experimental measures including height and bodyweight, and then followed by brachial and central blood pressure and arterial stiffness while I am in a supine position on the

health over the long term remains in question, but are not likely to be of harm from a one time participation in this study.

Discomfort during blood pressure assessment: It is noteworthy that the squeezing of the infatable cuff on the body during assessments of blood pressure can cause very slight discomfort but will be alleviated after a couple of seconds.

Musculoskeletal injury from exercise: performing moderate intensity exercise poses risk for injury to the musculoskeletal systems or possible death, but this risk is considered to be trivial and the health benefits of exercise far outweigh the risks. I will be familiarized to the use of a treadmill if I have had little past exposure to using such equipment.

Time Commitment: Although participation in the study will require considerable time commitment (15-16 hours over a course of 4 visits), participants will be allowed and encouraged to complete school or remote work during the prolonged sitting interventions.

5. **Confidentiality:**

Information produced by this study will be stored in the investigator's file and de-identified by a code number only. The code key connecting my name to specific information about me will be kept in a separate, secure location. Information contained in my records may not be given to anyone unaffiliated with the study in a form that could identify you without your written consent, except as required by law. It is possible that my medical and/or research record, including sensitive information and/or identifying information, may be inspected and/or copied by the study sponsor and/or federal or state government agencies in the course of carrying out their duties. If my record is inspected or copied by the study sponsor (and/or its agents), or by any of these agencies, the University of Southern Mississippi will use reasonable efforts to protect my privacy and the confidentiality of my medical information. The results of this study may be published in a medical book or journal or used for teaching purposes. However, my name or other identifying information will not be used in a publication or teaching materials without my specific permission.

6. **Alternative Procedures:**
An alternative procedure is to not participate in this research study.

7. **Participant's Assurance:**
This project and this consent form have been reviewed by USM's Institutional Review Board, which ensures that research projects involving human subjects follow federal regulations. Any questions or concerns about rights as a research participant should be directed to the Chair of the Institutional Review Board, The University of Southern Mississippi, 118 College Drive #5125, Hattiesburg, MS 39406-0001, 601-266-5997.

Any questions about this research project should be directed to the Principal Investigator using the contact information provided above.

CONSENT TO PARTICIPATE IN RESEARCH

Participant's Name: _____

I hereby consent to participate in this research project. All research procedures and their purpose were explained to me, and I had the opportunity to ask questions about both the procedures and their purpose. I received information about all expected benefits, risks, inconveniences, or discomforts, and I had the opportunity to ask questions about them. I understand my participation in the project is completely voluntary and that I may withdraw from the project at any time without penalty, prejudice, or loss of benefits. I understand the extent to which my personal information will be kept confidential. As the research proceeds, I understand that any new information that emerges and that might be relevant to my willingness to continue my participation will be provided to me.

(Include the following information only if applicable. Otherwise delete this entire paragraph before submitting for IRB approval:) The University of Southern Mississippi has no mechanism to provide compensation for participants who may incur injuries as a result of participation in research projects. However, efforts will be made to make available the facilities and professional skills at the University. Participants may incur charges as a result of treatment related to research injuries. Information regarding treatment or the absence of treatment has been given above.

_____	_____
Research Participant	**Person Explaining the Study**
_____	_____
Date	**Date**

Appendix F - Medical History Form

Medical History Form **Page 2**

Alcohol Consumption History:

Do you currently drink alcohol?	If you drank alcohol previously , when did you stop?
If you ever did drink alcohol, what is (was) the volume consumed? _____ # ounces / day for _____ # of years	

Exercise History:

Do you currently exercise aerobically?	How many years?	Duration:
	Types of Exercise:	Frequency:
Do you compete in endurance events?	How many years?	Frequency:
	What events?	
If you are currently sedentary, when did you last exercise?	How many years?	Duration:
	Types of Exercise:	Frequency:

Medical History:

NO	YES	Please explain any "YES" answers
		high blood pressure
		chest pain / history of heart attack
		extra heart beats or racing
		abnormal electrocardiogram (ECG)
		other heart trouble (eg murmur, valve problems)
		high cholesterol
		diabetes
		seizures
		stroke
		fainting spells
		anxiety (diagnosed)
		depression (diagnosed)
		recurrent fatigue
		insomnia
		thyroid problems
		difficulty breathing
		emphysema/ asthma/ chronic bronchitis
		tuberculosis
		chronic infection
		stomach/GI problems
		hepatitis
		bleeding disorder
		kidney/ urinary problems
		joint injuries/ joint pain
		arthritis (rheumatoid or osteoarthritis)
		migraine headaches
		vision problems (exclude corrected near/far sightedness)
		surgical procedures

Please sign and date.

Signature: _____ **Date:** _____

Medical History Form

All of the information provided in this form is voluntary.

Date: Biographical information:

Last Name First MI

Occupation: Email:

Home Phone( ) Work ( ) Cell/Pager ( )

Address:

DOB: / / Age: Gender M / F Height: Weight:

Highest Education Achieved:

Race: What race do you consider yourself to be? Select one or more of the following:

- ☐ ***Hispanic or Latino*** - A person of Mexican, Puerto Rican, South or Central American, or other Spanish culture or origin, regardless of race. The term "Spanish origin," can be used in addition to "Hispanic or Latino."
- ☐ **American Indian or Alaska Native.** A person having origins in any of the original peoples of North, South, or Central America, and who maintains a tribal affiliation or community attachment.
- ☐ **Asian.** A person having origins in any of the original peoples of the Far East, Southeast Asia, or the Indian subcontinent, including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam. (Note: Individuals from the Philippine Islands have been recorded as Pacific Islanders in the previous data collection strategies.)
- ☐ **Black or African American.** A person having either origins in any of the black racial groups of Africa. Terms such as "Haitian" or "Negro" can be used in addition to "Black" or "African American."
- ☐ **Native Hawaiian or Pacific Islander.** A person having origins in any of the original peoples of Hawaii, Guam, Samoa, or other Pacific islands.
- ☐ **White.** A person having origins in any of the original peoples of Europe, the Middle East, or North Africa.

Primary Care Physician:

Name: Office Phone:

Address:

Emergency Contact:

Name: Relationship: Phone #

Medications: include over the counter drugs/oral contraceptives/dietary supplements

Name/Dosage/How often taken:

Allergies:

Smoking History:

Do you smoke Cigarettes? Pipe/ Cigar? Other? If you quit, what year did you quit _____

# of packs smoked per day _____ For how many years _____

Appendix G – Protocol Checklist

PARTICIPANT VISIT CHECKLIST

- ☐ Informed Consent
- ☐ Medical History
- ☐ Questionnaires (PA)
- ☐ Needs to Meet with Principal Investigator

The assessments must be completed in the following order by the individuals listed. Under no circumstances is this protocol to be altered unless approved by the Principal Investigator for this participant.

INITIALS	TASK (First Visit)
	Greet Participant
	Temperature, Mask and Symptoms Confirmation
	Height and Weight
	Instrumentation of Participant
	Measurements for PWV
----	Pre-Intervention Measurements (Supine)
	Brachial Blood Pressure **(2 Measures)**
	Central Blood Pressure **(2 Measures)**
	PWV **(2 Measures)**
	Resting Heart Rate **(2 Measures)**
----	During-Intervention Measurements
	Brachial Blood Pressure at 60 min **(2 Measures)**
	Central Blood Pressure at 60 min **(2 Measures)**
	Brachial Blood Pressure at 120 min **(2 Measures)**
	Central Blood Pressure at 120 mins **(2 Measures)**
----	Post-Intervention Measurements
	Brachial Blood Pressure **(2 measures)**
	Central Blood Pressure **(2 Measures)**
	PWV **(2 Measures)**
----	Save all participant files
	Brachial Blood Pressure **(2 Measures)**
	Central Blood Pressure (PWA) **(2 Measures)**
	PWV **(2 Measures)**
	Clean and Disinfect Laboratory and Equipment

Appendix H – Protocol Data Recording Sheet

Intermittent Standing and Prior Exercise as Strategies to Prevent Arterial Stiffening That Car Arise from Prolonged Sitting

Participant ID: STA __ __
Lab ID: __ __
Date of Evaluation: ____ / ___ / _

SECTION I: STUDY VISIT INFORMATION

Study Visit	SO
Room Temperature	
Most Recent Meal *2 Hours Fasted*	
Caffeine Consumption?	
Mod-Vig PA?	
Tobacco or Alcohol?	

NOTES:

SECTION II: DEMOGRAPHICS

Gender	
DOB (m/d/yr)	
Age (years)	
Race	

SECTION III: HEIGHT AND WEIGHT ASSESSMENT

Height (cm)*	
Weight (kg)**	

*Body Height: measured to the nearest 0.1 cm
**Body Weight: measured to the nearest 0.1 k

SECTION V: INSTRUMENTATION

- ☐ Start timer once participant lies down (5 minutes)
- ☐ Attach SphygmoCor Cuffs (arm and thigh)
- ☐ Measurements for PWV (mm)

Carotid to Suprasternal Notch		Suprasternal Notch to Femoral Cuff	

Intermittent Standing and Prior Exercise as Strategies to Prevent Arterial Stiffening That Can Arise from Prolonged Sitting

Participant ID: STA __ __ __
Lab ID: __ __ __
Date of Evaluation: ____ / ___ / ____

Condition:

SECTION VI: PRE-INTERVENTION MEASURES (SUPINE)		Record Time:
Brachial Blood Pressure		mm/Hg
		mm/Hg
		mm/Hg
PWA – Central Pressure		mm/Hg
		mm/Hg
		mm/Hg
Aortic-PWV (swap cuff)		m/sec
		m/sec
		m/sec
SECTION VII: DURING-INTERVENTION MEASURES		**Record Time:**
Brachial Blood Pressure (60 MINS)		mm Hg
		mm/Hg
		mm Hg
PWA – Central Pressure (60 MINS)		mm Hg
		mm/Hg
		mm Hg
Brachial Blood Pressure (120 MINS)		mm Hg
		mm/Hg
		mm Hg
PWA – Central Pressure (120 MINS)		mm Hg
		mm/Hg
		mm Hg
SECTION VII: POST-INTERVENTION MEASURES (SUPINE)		**Record Time:**
Transfer Participant to examination table using mechanical lift		mm/Hg
Brachial Blood Pressure		mm/Hg
		mm/Hg
		mm/Hg
PWA – Central Pressure		mm/Hg
		mm/Hg
		mm/Hg
Aortic-PWV (swap cuff)		m/sec
		m/sec
		m/sec

Appendix I – 2021 PAR-Q+ Form

2021 PAR-Q+

The Physical Activity Readiness Questionnaire for Everyone

The health benefits of regular physical activity are clear; more people should engage in physical activity every day of the week. Participating in physical activity is very safe for MOST people. This questionnaire will tell you whether it is necessary for you to seek further advice from your doctor OR a qualified exercise professional before becoming more physically active.

GENERAL HEALTH QUESTIONS

Please read the 7 questions below carefully and answer each one honestly: check YES or NO.	YES	NO
1) Has your doctor ever said that you have a heart condition ☐ **OR** high blood pressure ☐?	☐	☐
2) Do you feel pain in your chest at rest, during your daily activities of living, **OR** when you do physical activity?	☐	☐
3) Do you lose balance because of dizziness **OR** have you lost consciousness in the last 12 months? Please answer **NO** if your dizziness was associated with over-breathing (including during vigorous exercise).	☐	☐
4) Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)? **PLEASE LIST CONDITION(S) HERE:**	☐	☐
5) Are you currently taking prescribed medications for a chronic medical condition? **PLEASE LIST CONDITION(S) AND MEDICATIONS HERE:**	☐	☐
6) Do you currently have (or have had within the past 12 months) a bone, joint, or soft tissue (muscle, ligament, or tendon) problem that could be made worse by becoming more physically active? Please answer **NO** if you had a problem in the past, but it **does not limit your current ability** to be physically active. **PLEASE LIST CONDITION(S) HERE:**	☐	☐
7) Has your doctor ever said that you should only do medically supervised physical activity?	☐	☐

If you answered NO to all of the questions above, you are cleared for physical activity.
Please sign the PARTICIPANT DECLARATION. You do not need to complete Pages 2 and 3.

- Start becoming much more physically active – start slowly and build up gradually.
- Follow Global Physical Activity Guidelines for your age (https://www.who.int/publications/i/item/9789240015128).
- You may take part in a health and fitness appraisal.
- If you are over the age of 45 yr and NOT accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.
- If you have any further questions, contact a qualified exercise professional.

PARTICIPANT DECLARATION

If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that the community/fitness center may retain a copy of this form for its records. In these instances, it will maintain the confidentiality of the same, complying with applicable law.

NAME _____ DATE _____

SIGNATURE _____ WITNESS _____

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER _____

If you answered YES to one or more of the questions above, COMPLETE PAGES 2 AND 3.

Delay becoming more active if:

- You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ at www.eparmeds.com before becoming more physically active.
- Your health changes - answer the questions on Pages 2 and 3 of this document and/or talk to your doctor or a qualified exercise professional before continuing with any physical activity program.

 1 / 4

01-11-2020

2021 PAR-Q+

FOLLOW-UP QUESTIONS ABOUT YOUR MEDICAL CONDITION(S)

1. Do you have Arthritis, Osteoporosis, or Back Problems?

If the above condition(s) is/are present, answer questions 1a-1c — If **NO** ☐ go to question 2

1a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
1b.	Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebra (e.g., spondylolisthesis), and/or spondylolysis/pars defect (a crack in the bony ring on the back of the spinal column)?	YES ☐ NO ☐
1c.	Have you had steroid injections or taken steroid tablets regularly for more than 3 months?	YES ☐ NO ☐

2. Do you currently have Cancer of any kind?

If the above condition(s) is/are present, answer questions 2a-2b — If **NO** ☐ go to question 3

2a.	Does your cancer diagnosis include any of the following types: lung/bronchogenic, multiple myeloma (cancer of plasma cells), head, and/or neck?	YES ☐ NO ☐
2b.	Are you currently receiving cancer therapy (such as chemotheraphy or radiotherapy)?	YES ☐ NO ☐

3. Do you have a Heart or Cardiovascular Condition? This includes Coronary Artery Disease, Heart Failure, Diagnosed Abnormality of Heart Rhythm

If the above condition(s) is/are present, answer questions 3a-3d — If **NO** ☐ go to question 4

3a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
3b.	Do you have an irregular heart beat that requires medical management? (e.g., atrial fibrillation, premature ventricular contraction)	YES ☐ NO ☐
3c.	Do you have chronic heart failure?	YES ☐ NO ☐
3d.	Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months?	YES ☐ NO ☐

4. Do you currently have High Blood Pressure?

If the above condition(s) is/are present, answer questions 4a-4b — If **NO** ☐ go to question 5

4a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
4b.	Do you have a resting blood pressure equal to or greater than 160/90 mmHg with or without medication? (Answer **YES** if you do not know your resting blood pressure)	YES ☐ NO ☐

5. Do you have any Metabolic Conditions? This includes Type 1 Diabetes, Type 2 Diabetes, Pre-Diabetes

If the above condition(s) is/are present, answer questions 5a-5e — If **NO** ☐ go to question 6

5a.	Do you often have difficulty controlling your blood sugar levels with foods, medications, or other physician-prescribed therapies?	YES ☐ NO ☐
5b.	Do you often suffer from signs and symptoms of low blood sugar (hypoglycemia) following exercise and/or during activities of daily living? Signs of hypoglycemia may include shakiness, nervousness, unusual irritability, abnormal sweating, dizziness or light-headedness, mental confusion, difficulty speaking, weakness, or sleepiness.	YES ☐ NO ☐
5c.	Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, **OR** the sensation in your toes and feet?	YES ☐ NO ☐
5d.	Do you have other metabolic conditions (such as current pregnancy-related diabetes, chronic kidney disease, or liver problems)?	YES ☐ NO ☐
5e.	Are you planning to engage in what for you is unusually high (or vigorous) intensity exercise in the near future?	YES ☐ NO ☐

2021 PAR-Q+

6. **Do you have any Mental Health Problems or Learning Difficulties?** *This includes Alzheimer's, Dementia, Depression, Anxiety Disorder, Eating Disorder, Psychotic Disorder, Intellectual Disability, Down Syndrome*

If the above condition(s) is/are present, answer questions 6a-6b. If **NO** ☐ go to question 7

6a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
6b.	Do you have Down Syndrome **AND** back problems affecting nerves or muscles?	YES ☐ NO ☐

7. **Do you have a Respiratory Disease?** *This includes Chronic Obstructive Pulmonary Disease, Asthma, Pulmonary High Blood Pressure*

If the above condition(s) is/are present, answer questions 7a-7d. If **NO** ☐ go to question 8

7a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
7b.	Has your doctor ever said your blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy?	YES ☐ NO ☐
7c.	If asthmatic, do you currently have symptoms of chest tightness, wheezing, laboured breathing, consistent cough (more than 2 days/week), or have you used your rescue medication more than twice in the last week?	YES ☐ NO ☐
7d.	Has your doctor ever said you have high blood pressure in the blood vessels of your lungs?	YES ☐ NO ☐

8. **Do you have a Spinal Cord Injury?** *This includes Tetraplegia and Paraplegia*

If the above condition(s) is/are present, answer questions 8a-8c. If **NO** ☐ go to question 9

8a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
8b.	Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting?	YES ☐ NO ☐
8c.	Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as Autonomic Dysreflexia)?	YES ☐ NO ☐

9. **Have you had a Stroke?** *This includes Transient Ischemic Attack (TIA) or Cerebrovascular Event*

If the above condition(s) is/are present, answer questions 9a-9c. If **NO** ☐ go to question 10

9a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
9b.	Do you have any impairment in walking or mobility?	YES ☐ NO ☐
9c.	Have you experienced a stroke or impairment in nerves or muscles in the past 6 months?	YES ☐ NO ☐

10. **Do you have any other medical condition not listed above or do you have two or more medical conditions?**

If you have other medical conditions, answer questions 10a-10c. If **NO** ☐ read the Page 4 recommendations

10a.	Have you experienced a blackout, fainted, or lost consciousness as a result of a head injury within the last 12 months **OR** have you had a diagnosed concussion within the last 12 months?	YES ☐ NO ☐
10b.	Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, kidney problems)?	YES ☐ NO ☐
10c.	Do you currently live with two or more medical conditions?	YES ☐ NO ☐

PLEASE LIST YOUR MEDICAL CONDITION(S) AND ANY RELATED MEDICATIONS HERE: _____

_____

GO to Page 4 for recommendations about your current medical condition(s) and sign the PARTICIPANT DECLARATION.

2021 PAR-Q+

If you answered NO to all of the FOLLOW-UP questions (pgs. 2-3) about your medical condition, you are ready to become more physically active - sign the PARTICIPANT DECLARATION below:

- It is advised that you consult a qualified exercise professional to help you develop a safe and effective physical activity plan to meet your health needs.
- You are encouraged to start slowly and build up gradually - 20 to 60 minutes of low to moderate intensity exercise, 3-5 days per week including aerobic and muscle strengthening exercises.
- As you progress, you should aim to accumulate 150 minutes or more of moderate intensity physical activity per week.
- If you are over the age of 45 yr and **NOT** accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.

If you answered **YES** to **one or more of the follow-up questions** about your medical condition:

You should seek further information before becoming more physically active or engaging in a fitness appraisal. You should complete the specially designed online screening and exercise recommendations program - the **ePARmed-X+ at www.eparmedx.com** and/or visit a qualified exercise professional to work through the ePARmed-X+ and for further information.

Delay becoming more active if:

- You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ **at www.eparmedx.com** before becoming more physically active.
- Your health changes - talk to your doctor or qualified exercise professional before continuing with any physical activity program.

- You are encouraged to photocopy the PAR-Q+. You must use the entire questionnaire and NO changes are permitted.
- The authors, the PAR-Q+ Collaboration, partner organizations, and their agents assume no liability for persons who undertake physical activity and/or make use of the PAR-Q+ or ePARmed-X+. If in doubt after completing the questionnaire, consult your doctor prior to physical activity.

PARTICIPANT DECLARATION

- All persons who have completed the PAR-Q+ please read and sign the declaration below.
- If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that the community/fitness center may retain a copy of this form for records. In these instances, it will maintain the confidentiality of the same, complying with applicable law.

NAME _____ DATE _____

SIGNATURE _____ WITNESS _____

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER _____

For more information, please contact

www.eparmedx.com

Email: eparmedx@gmail.com

Citation for PAR-Q+

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 4/ 4

Appendix J – Godin Leisure-Time Exercise Questionnaire

Godin Leisure-Time Exercise Questionnaire

1. During a typical **7-Day period** (a week), how many times on the average do you do the following kinds of exercise for **more than 15 minutes** during your free time (write on each line the appropriate number).

	Times Per Week
a) **STRENUOUS EXERCISE** **(HEART BEATS RAPIDLY)** (e.g., running, jogging, hockey, football, soccer, squash, basketball, cross country skiing, judo, roller skating, vigorous swimming, vigorous long distance bicycling)	_____
b) **MODERATE EXERCISE** **(NOT EXHAUSTING)** (e.g., fast walking, baseball, tennis, easy bicycling, volleyball, badminton, easy swimming, alpine skiing, popular and folk dancing)	_____
c) **MILD EXERCISE** **(MINIMAL EFFORT)** (e.g., yoga, archery, fishing from river bank, bowling, horseshoes, golf, snow-mobiling, easy walking)	_____

2. During a typical **7-Day period** (a week), in your leisure time, how often do you engage in any regular activity **long enough to work up a sweat** (heart beats rapidly)?

OFTEN	SOMETIMES	NEVER/RARELY
1. ☐	2. ☐	3. ☐

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